BLOOD GROUP GENOTYPING: THE FUTURE IS NOW

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• “...a gene had been defined as a region of the genome that segregates as a single unit during meiosis and gives rise to a definable phenotypic trait”.

• “...a gene became identified as that stretch of DNA that was transcribed into the RNA coding for a single polypeptide chain”

A Gene at the Molecular Level
mRNA is Transcribed
Into a Gene Product

DNA

Intron

Splicing

Exon

mRNA
Typing for Blood Group Genes

- Traditionally has been done by phenotyping using serological methods
- Can now be done by genotyping (DNA)
  - PCR-RFLP
  - PCR-SSP or AS-PCR
  - Real-time PCR
  - Sequencing
  - Microarray
Molecular Typing Terminology

- **PCR**: polymerase chain reaction
  - = amplification of a gene a million-fold
- **Primers**: a string of ~20 nucleotides that are complementary to the gene being amplified
- **Multiplex PCR**: amplification of more than one gene in a single reaction
- **SNP**: single nucleotide polymorphism
Thermal Cycler for PCR
Polymerase Chain Reaction (PCR)

- **Template**
- **Melt DNA**
- **Anneal Primers**
- **Extension**
- **Products**
Restriction Fragment Length Polymorphism (RFLP)

- **Restriction enzymes:** named after the bacteria in which they are found
  - *Hin* III, *Eco* RI
- PCR product is digested with enzymes
- Fragments are separated by gel electrophoresis
Allele Specific PCR

- Primer is designed only to detect one allele of a gene
- Must set up multiple PCR reactions to detect 2 or more alleles as well as a DNA control
DNA Sequencing

- 1st do a PCR with primers for the gene of interest
- “clean-up” PCR product
- Set-up sequencing reaction and run gel (automated)
- Good if several SNPs are in the same region
Microarray Genotyping

- Oligonucleotide probes on glass slides
- ABO, Rh (Cc, D, Ee)
- Kell, Jk, Fy, Di, Do, Co, MNSs
Bead-Chip Microarray

- **Type for:** C/c, E/e, K1/K2, MNSs, J KA/J KB, FYA/FYB, DIA/DIB, COA/COB, LWA/LWB, SC1/SC2, DOA/DOB, HY, J O, LUA/LUB, Hgb S
- **Cost is competitive due to saving of tech time**
MAKING BEADCHIPS ...
DNA ANALYSIS BY BEAD CHIP

DNA Extraction

↓

m’plex PCR

↓

post-PCR Processing

↓

BeadChip™ Analysis
96 sample results
x 28 antigens
DISCRIMINATION BY ALLELE-SPECIFIC ELONGATION

Assay Image

MATCH

DNA Polymerase

eMAP™
RECORDING & DECODING ARRAY IMAGES

Automated “Snapshot” Array Imaging

Decoding Image

Assay Image
ANALYZING DATA

Chip Analysis
Bar Chart

DNA Analysis
Δ Chart
(for each probe pair)

SNP = FY-GATA (FY -33T>C)
| ChipName       | Sample | WarnMsg | C | c | e | E | K | k | Fya | Fyb | Jka | Jkb | M | N | S | s | Lua | Lub | Doa | Dob | Jo(s) | Hy | LWa | LWb | Dia | Dib | Coa | Cob | Sc1 | Sc2 | HgbS | Silencing FY | Fyx(b+w) | Cmmt |
|---------------|--------|---------|---|---|---|---|---|---|-----|-----|-----|-----|---|---|---|---|-----|-----|-----|-----|--------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| HEA04961_1_1 | A1     |         | + | + | - | + | + | - | - | + | - | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 1 | No |
| HEA04961_2_2 | A2     |         | + | - | + | - | - | + | + | + | - | + | + | - | + | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | No | No |
| HEA04961_3_3 | A3     |         | + | + | + | - | + | + | + | - | - | + | + | - | + | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 1 | No |
| HEA04961_4_4 | A4     |         | + | - | + | - | - | + | - | - | + | + | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 1 | No |
| HEA04961_5_5 | A5     |         | + | + | + | - | - | + | + | + | - | + | + | - | + | + | - | - | + | + | + | + | - | + | - | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | 1 | No |
| HEA04961_6_6 | A6     |         | - | + | + | - | + | - | + | - | + | - | + | + | + | - | - | + | + | - | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 1 | No |
| HEA04961_7_7 | A7     |         | - | - | + | - | + | + | - | - | + | + | - | + | + | + | - | - | + | + | - | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | No | No |
| HEA04961_8_8 | A8     |         | - | + | + | - | + | - | - | + | + | - | + | + | + | - | - | + | + | - | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | No | No |
Why do DNA Typing?

- To identify at risk fetus & paternal zygosity
- To determine antigen status in problem cases
  - Recently transfused patient
  - Patient with positive DAT
  - Multiply transfused patients, eg. SCD
- To screen for antigen negative donors
  - When reagents or licensed antisera are unavailable for mass screening
DNA Typing Commercial Donors

- Antigen type when suitable antisera is unavailable eg. Do^b, J s^a, V/VS

- To confirm homozygosity
  - 3 RhD samples were heterozygous Dd

- Confirm weak antigens
  - 1 Fy(a+b-) sample was FY*A/FY*X (Fy^b weak)
So Now Let’s Peek Into Your Genes

- Some LifeShare experiences.....
Evaluation & Validation of the Bead-Chip Microarray

- Tested 84 rare or null phenotypes:
  - e-, k-, Jk-3, Fy(a-b-), U-, Lu(b-), Di(b-), Co(a-), Hy-, Jo(a-), LW(a-), Sc-1
- Tested 40 Black donors in duplicate:
  - In house for precision
  - Another BAS user for accuracy
- Concurrent testing of 200 random Blacks:
  - by serology for C/c, E/e, MNSs, Fy, Jk, Kell
  - by DNA for 28 antigens
Screening for Rare Donors

- Patient had anti-Hy
  - Diluted 1:40 for gel
  - Negatives confirmed with undiluted antisera

- Serologically typed 3,443 random group

O Black donors:
  10 Hy- in 18 months

- Genotyped ~800
  3 Hy- in 2 months

Area screened for Hy-
In the First 500 Blacks
We Also Found........

- **Rares**
  - 10 R2R2 (e neg)
  - 7 U neg
  - 4 Co(a-b+)
  - 4 Hy-
  - 3 Jo(a-)
  - 1 Sc -1,2

- **Unusual**
  - 14 Co(a+b+)
  - 17 Lu(a+b+)
  - 6 Sc 1,2
  - 1 Di(a+)
Screening for Phenotype Matched Donors

- 450 units supplied to ARDP in 2006
- 97% were phenotype matched for Rh, Kell, Duffy, Kidd, MNSs
- NIH recommendation for transfusion of SCD patients:
  - Minimum of C/c, E/e, K1 matched
  - If possible do Fy, Jk, MNSs
- Can we match even better?
COMPATIBLE BLOOD: 16 ANTIGEN-MATCH EXAMPLE

NYC ETHNICITY PROFILE

SUPPLY

DEMAND

COMPATIBLE UNITS

% FULFILLMENT

Note: optimal “firing sequence”: i.e., 1ST priority in all cases to Black patients
16 Antigen base = Fya /Fyb /Lua /Lub /M /N /S /s /K /k /Jka /Jkb /Doa /Dob /Hy /Joa
Other Uses for Genotyping

- Recently transfused patients who develop alloantibodies
- Patients with strongly positive DAT
  DAT = 3 to 4+
  Unable to remove with chloroquine or EGA
  Strong serum antibody, unable to absorb
  *Perform genotype and give matched units*
- Reagent red cells (home grown)
Duffy Typing Discrepancy

- Unit labeled on the shelf as Fy(a-b-)
- Genotyping by bead-chip microarray
- Homozygous FY*B gene but
  - heterozygous for -33 (GATA) SNP
  - “ “ 265 SNP
  - FY*FY/FY*X or Fy(a-b+w)
Technology is now available for blood group genotyping
- On an individual case
- For high throughput typing

We now can provide the best product for our repeat customers