Genetics and Genetic testing

Jane Halliday
Public Health Genetics, MCRI
Context

- Genetic testing occurs in many settings at all stages of life
- Genetic Health Services Victoria here at RCH
  - Clinical service to provide a diagnosis of a chromosomal or single gene disorder in affected individual:
  - Carrier testing in families at risk
  - Population based testing
    - Established screening programs
      - Prenatal
      - Newborn
      - School age children (Tay Sachs, CF)
Outline of talk

Established programs
1. Prenatal screening/diagnosis for chromosome abnormalities
2. Newborn screening for single gene defects

New program
3. Infant/childhood screening for chromosomal abnormalities

Future possibilities
What are genetic tests testing for?

- Chromosomal abnormalities
  - Aneuploidy (numerical differences)
  - Deletions, duplications, translocations (structural differences, imbalances)
- Gene changes
  - Single gene mutations
  - Single nucleotide polymorphisms
  - Copy number variations
What causes a congenital abnormality / birth defect?

- **Genetic (15-25%)** eg. extra chromosome, deletion, new mutation, inherited Mendelian disorders

- **Environmental (10%)**
  - *mechanical probs* - deformations (1-2%), amniotic band constrictions, umbilical cord
  - *maternal conditions* (4%) - alcoholism, diabetes, PKU, nutritional deficits
  - *infectious agents* (3%) - rubella, toxo, CMV
  - *chemicals* (<1%), drugs, radiation, hyperthermia

- **Unknown (65-75%)** - polygenic, multifactorial, spont errors in development, synergistic interactions of teratogens

**EPIGENETIC**

*Brent RL. Pediatrics 2004: 113;957-968*
1. Prenatal testing for chromosome abnormalities

47,XY,+21 or Down Syndrome
Prenatal testing in Victoria:

1. Diagnostic testing

- Late 1970s: Prenatal diagnosis
- 1980s: "Routine" anomaly ultrasound
- Late 1970s prenatal diagnosis
- Amnio
Prenatal testing in Victoria

2. Screening

1986 CVS
1980s "routine" anomaly ultrasound
late 1970s prenatal diagnosis Amnio

1996-1997 2nd trimester Mat Serum Screening
1997 nuchal translucency ultrasound
2000 1st trimester combined screening

A SIMPLE BLOOD TEST WILL TELL

The maternal serum test is a blood test for pregnant women to find out if they have an increased risk of having a baby with birth defects such as Down syndrome and spina bifida.

The blood test measures levels of four pregnancy chemicals in relation to these conditions. Women who are identified as high risk are offered further testing such as ultrasound or amniocentesis.

Counselling to discuss the test result and its implications is available from the Royal Children’s Hospital.

All pregnant women are free to take or refuse the serum test.

For more information, ask your doctor or midwife or contact the Victorian Clinical Genetics Services on 9845 5157.

Source: Royal Women’s Hospital Health Information Centre.

A maternal serum test at the Royal Children’s Hospital.
Uptake of prenatal testing

Number of tests

- 1TCS
- CVS
- 2TMSS
- Amnio

L. Bonacquisto, and E. Muggli, *Personal Communications*
Reasons for prenatal diagnosis in 2008

- First trimester combined screen*: 46%
- Advanced maternal age: 28%
- Abnormal ultrasound^: 14%
- Second trimester maternal serum screen*: 14%
- Chromosome abnormality#: 14%
- Single gene disorder#: 4%
- Chromosome rearrangement/translocation#: 4%
- Repeat test: 4%
- Other (within HGSA/RANZCOG guidelines): 4%
- Outside HGSA/RANZCOG guidelines: 4%
Proportion of all tests that have abnormal karyotype

Number of trisomies

Year

Trisomy 13    Trisomy 18    Trisomy 21

Year

Number of trisomies

Proportion of all tests that have abnormal karyotype

3% 9%

Year

96 98 00 02 04 06 08
A family’s most painful decision

Deciding to have an abortion is traumatic. It is all the more difficult when protesters confront the mother.

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SUNDAY HERALD SUN SUNDAY MAGAZINE SUNDAY 31 AUGUST 2003

SUNDAY AGE AGENDA SUNDAY 18 JULY 2004

Since her daughter was born with Down syndrome one year ago life has been a blur of medical appointments, exhaustion and anxiety for Kathy Evans. But she wouldn’t change it for the world.

THE AGE FRIDAY 14 AUGUST 1998

THE AGE WEDNESDAY 15 JULY 1998

THE AGE FRIDAY 14 AUGUST 1998

THE SUNDAY AGE AGENDA SUNDAY 18 JULY 2004

DOWN SYNDROME: THE FACTS

Down syndrome is a chromosomal disorder in which some or all of the cells in the body contain an extra chromosome.

- Each cell in the human body normally contains 46 chromosomes, existing in 23 pairs. Individuals with Down syndrome, however, have three copies of chromosome 21 rather than two (the syndrome is also known as Trisomy 21). What causes this replication is still unknown. No racial, geographical, social, economic or environmental factors have been identified.

- Down syndrome is one of the most common genetic disorders, affecting one in 600-700 live births worldwide. While the risk of Down syndrome increases with the age of the mother, most children with Down syndrome are born to younger parents.

- While the additional chromosome appears to carry normal genes, its repetition results in a set of unique physical and medical characteristics. People with Down syndrome may have almond-shaped eyes, be shorter than their peers, have smaller mouths, ears, hands and feet, and have low muscle tone. But because they carry genes from their parents, people with Down syndrome also bear family characteristics.

- Individuals with Down syndrome are also at risk of a number of medical conditions such as heart defects, thyroid problems and digestive obstructions. Mild medical conditions such as ear infections and colds usually occur more frequently. But because of improved medical care the life span of individuals with Down syndrome has increased – the average life expectancy is now 65-plus, with some people living into their 80s.

- People with Down syndrome are usually mildly or moderately intellectually disabled. Research suggests that while there is great variation among individuals, the potential range of ability and achievement is higher than previously believed. Traditional assumptions about the capabilities of people with Down syndrome are being challenged by changes in early intervention and education.

- While they may need additional support, many children with Down syndrome are now attending normal primary and high schools. Adults with Down syndrome live independently and hold down jobs in the community.

National Down Syndrome Awareness Week runs from October 12 to 19. Information provided by the Down Syndrome Association of Victoria: (03) 9486 2377.
Providing decision support

NHMRC-funded Randomised Controlled Trial

Development and evaluation of Decision Aid related to prenatal screening for fetal abnormality, to be disseminated by GPs. Jane Halliday, Jane Gunn, Robin Bell, Bettina Meiser, Sylvia Metcalfe, John Carlin

Decision aid arm vs pamphlet arm

Primary health care
Setting: 50 GPs, each to recruit 10 women at 1st visit

Cluster design used to minimise contamination between groups
Decision Aid

24 page booklet featuring:

Flexible format using contents page, summary tables, dot points and resources

Individualised age related risk for Down syndrome, use of pie charts for representation of risk, positive and negative framing

Hypothetical scenarios of women making PNT decisions—women’s stories
Adjust for practice location, gender of GP, years of general practice, maternal age, woman’s highest education level, woman’s religion, previous termination of pregnancy and previously undertaken screening tests

Nagle C, Gunn J, Bell R, Lewis S, Meiser B, Metcalfe S, Ukoumunne O and Halliday J.
A Decision Aid

A new patient education publication from the College and the Murdoch Childrens Research Institute

- Enhance doctor-patient communications
- Assist the informed-consent process

The aim of the Decision Aid is to assist women in early pregnancy to make decisions regarding testing for fetal abnormalities.

Written in plain English and reviewed extensively by the College and the MCRI, the Decision Aid is an eight-page full-colour pamphlet with anatomical illustrations that covers key issues in:
- Chromosomal abnormalities
- Explanations of "low risk" and "increased risk"
- Fetal transvaginal ultrasound
- Maternal serum screening – first trimester
- Maternal serum screening – second trimester
- Chorionic villus sampling
- Amniocentesis
- Practical stories of women’s decisions
- Work sheets to assist with decision-making
- Costs and matters about informed consent.

Joint signatories

Dear Doctor,

re: A Decision Aid – Testing in pregnancy for fetal abnormalities

For the benefit of Fellows, Diplomates and their patients, the Women’s Health Committee and the Murdoch Childrens Research Institute (MCRI) have produced a patient education pamphlet titled: A Decision Aid – Testing in pregnancy for fetal abnormalities. A copy is enclosed for your perusal.

Reviewed extensively by members of the Women’s Health Committee and MCRI, this edition of A Decision Aid was derived from an original document developed by the MCRI and collaborating healthcare professionals with funding from the National Health and Medical Research Council.

We believe this Decision Aid will help to answer the concerns of women and their families while providing obstetricians and gynaecologists with a comprehensive publication to enhance their communications with patients.

This Decision Aid and other RANZCOG patient education pamphlets are not intended to replace discussions between doctors and their patients, but rather to complement discussions and assist the informed-consent process.

We would request that you consider purchasing A Decision Aid for your patients. To receive copies for distribution to your patients, fill in the enclosed order form as indicated.

Yours sincerely,

[Signatures]

Ted Weaver
Chair
Women’s Health Committee

Jane Halliday
Associate Professor
Murdoch Childrens Research Institute

RANZCOG PATIENT EDUCATION PAMPHLETS

- Labour and its management
- Obesity during pregnancy
- Endoscopy of the pelvic organs
- Miscarriage
- Laparoscopy
- Understanding endometriosis
- Laparoscopic treatment of endometriosis
- Induction of labour
- Hysterectomy
- Abnormal pph smear
- Exercise during pregnancy
- Travel during pregnancy
- Hypertension during pregnancy
- Pain relief during childbirth

Joint signatories
A Decision Aid
Your choice: screening and diagnostic testing in pregnancy
more info on screening and diagnostic tests

Most maternity hospitals and ultrasound centres have pamphlets and online downloads explaining ultrasounds, maternal serum screening, amniocentesis and CVS. Ask your doctor, obstetrician, midwife or ultrasound operator for their info booklets.

Your Choice: Screening and Diagnostic Tests in Pregnancy by the Murdoch Childrens Research Institute, Australia.

An utterly brilliant booklet with tick-the-box responses to lead you through your own decisions about whether to take the tests available and how to understand and respond to results, based on your own feelings and philosophy. It explains all available tests and procedures, their advantages and risk. Compiled by a stellar list of Aussie medical and public health experts in pregnancy screening and diagnostic testing, and informed by the questions of pregnant women and their GPs. Download from: www.mcri.edu.au/Downloads/PrenatalTestingDecisionAid.pdf.


This constantly updated book is written by leading medical experts, one an Australian, the other from the US. It’s small but packed with useful medical and other details about the most common tests and abnormalities, what test results mean, common feelings, and how to make sure you get a good CVS or amnio operator. It answers lots of questions asked by real patients.
looking ahead...... non-invasive prenatal diagnosis
Non-invasive Prenatal Diagnosis (NIPD)

- NIPD has been described as the ‘Holy Grail’ of prenatal testing
- Cell free fetal DNA and RNA is present in maternal serum
- DNA-based testing used to measure ratio of chromosomes rather than karyotype
- **BUT** many issues to address
  - See papers by de Jong et al 2010; Greely, 2011
Summary of prenatal testing

Monitoring of testing (both screening and diagnosis) is recommended best practice and has been able to be done in Victoria for many years.

Prenatal screening has increased in popularity, while numbers of invasive prenatal diagnostic tests have declined.

Prenatal screening has ensured improved predictive value of prenatal diagnosis with a higher proportion of diagnostic tests detecting an abnormal karyotype.

Difficult issues to address in deciding whether or not to have a screening or diagnostic test and use of a Decision Aid may help.

Non-invasive diagnostic tests on the horizon.
2. Newborn Screening in Victoria for single gene defects

<table>
<thead>
<tr>
<th>Hospital Name and ward</th>
<th>[GREEN] - NEW BLOCK</th>
<th>UR/Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctor's name and initials</td>
<td>[RED] - NEW BLOCK</td>
<td>Infant's full name</td>
</tr>
<tr>
<td>Date of birth</td>
<td>Date of sample</td>
<td></td>
</tr>
<tr>
<td>Gestation:</td>
<td>Milk feeds</td>
<td></td>
</tr>
<tr>
<td>Relevant Family History</td>
<td>Collectors initials</td>
<td></td>
</tr>
</tbody>
</table>

Guthrie Card – newborn blood spots

Hatched area (\[\]) indicates safe areas for puncture site.
Urgency of Screening

A ‘well looking’ baby may quickly become critically ill.
Newborn Screening Program in Victoria

- Phenylketonuria 1:12,000
- Congenital Hypothyroidism 1: 3,500
- Cystic Fibrosis 1: 2,500

- Metabolic disorders 1: 6,000
<table>
<thead>
<tr>
<th>disorder</th>
<th>cause</th>
<th>indicated by</th>
<th>clinical signs if untreated</th>
<th>treatment/management</th>
<th>~ number of cases p/yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>congenital hypothyroidism</td>
<td>thyroid gland unable to produce thyroid hormones (T₃ &amp; T₄)</td>
<td>high level of thyroid stimulating hormone (TSH)</td>
<td>delayed physical and mental development</td>
<td>thyroid hormone supplements</td>
<td>20</td>
</tr>
<tr>
<td>cystic fibrosis</td>
<td>abnormal secretions in the body, in particular the lung and pancreas</td>
<td>elevated level of trypsinogen (enzyme)</td>
<td>impaired digestive and respiratory function; infections and decreased life span</td>
<td>dietary supplements; physiotherapy</td>
<td>20</td>
</tr>
<tr>
<td>amino acid disorders*</td>
<td>absence of particular enzymes that break down amino acids – these build up in the system and are toxic</td>
<td>high level of phenylalanine (amino acid)</td>
<td>developmental delay; intellectual impairment</td>
<td>dietary modifications</td>
<td>10</td>
</tr>
<tr>
<td>fatty acid oxidation disorders*</td>
<td>absence of particular enzymes that turn fat into energy – fatty acids build up in the blood</td>
<td>abnormal levels of various acylcarnitines (fat transport protein)</td>
<td>low blood sugar; poor feeding; seizures; sudden death</td>
<td>avoid prolonged fasting; dietary modification</td>
<td>8</td>
</tr>
</tbody>
</table>
Consent and storage issues

- Informed verbal parental consent needed or written refusal
- Kept for 2 years for QA at the lab
- Secure offsite storage indefinitely approved by DH and Public Records Office
- Access: QA, diagnosis (transport blood sample interstate), forensic ID, research (ethics)
Science Gold Mine, Ethical Minefield

“Health agencies launched a system 40 years ago to identify babies at risk. Now there are millions of blood samples in files that researchers want to access, raising public concern.”

JENNIFER COUZIN-FRANKEL 10 APRIL 2009 VOL 324 SCIENCE
Ministerial report produced in 2006 because of
- adverse media attention (consent, storage, use) (2004/5)
- health issues centre research (2005)

24 recommendations
- trial written consent for screening
- separate consent for screening and secondary use
Dept Health-funded project underway

- **WHAT** - trial written consent process in 4 Victorian hospitals
  - give parents more information
  - inform them of their rights and options

- **WHY** – research found no consistent approach for giving of information
- parents were found to:
  - have limited knowledge about the program, leading to complaints
  - lack awareness of storage and secondary access to cards
  - be unaware of their ability to refuse screening / retrieve the card
- health professionals in general:
  - had limited knowledge about expanded screening, storage/access of cards and correct sample collection techniques

- **AIM** - to strengthen the program, maintain high participation and reduce complaints
• written consent pilot project begun in Nov 08; ready to roll out now
• updated parent brochure, blue book postcard
• developed e-learning tool for midwives
• recruited 4 hospitals
  • Bendigo
  • Royal Women's
  • Sunshine
  • Bairnsdale
• started end of February 2010
Newborn screening— for the health of your baby

What happens after screening— a reminder!

After screening, some blood remains on your baby’s screening card. These cards are stored in the laboratory for two years for quality control and in case further testing is needed. After two years, the cards are securely stored indefinitely. Access to stored cards is tightly controlled and protected by state legislation. During storage, cards may be accessed for:

- **Clinical testing for your baby** – sometimes the newborn blood sample can be useful if your child develops an illness later in life. The sample may also be able to provide useful health information for other family members.

- **Developing new tests** – as technology improves, new tests can be developed so that more medical conditions can be identified in babies as part of the newborn screening program.

- **As requested by a court of law.**
In addition, a small number of cards may be used for health research, if parental permission was given for this purpose. This may include investigating conditions that affect newborns and young children, such as cerebral palsy, deafness, infections, metabolic disorders and certain cancers.

This research is done without using personal details or identifying information. If researchers want to use your personal details, you will be contacted for your permission.

After the two year period of laboratory storage, you can apply to have your baby’s card transferred to you. This request must be made in writing to the Newborn Screening Laboratory, located at the Royal Children’s Hospital in Melbourne. Consent from both parents will be required.

More information is available from:

- your midwife
- a newborn screening counsellor @ Victorian Clinical Genetics Service (03) 8341 6201
- the Department of Health, Ph 03 9096 5011 or email: newborn.screening@dhs.vic.gov.au
training midwives e-tool
Written consent process (brochure handout)

- Brochure delivered in postnatal period
- Midwife discusses, answers questions/concerns
- At sample collection the midwife
  - Completes front of the card as normal
  - Asks parent (either) to complete the section on the card & sign
  - Collects blood sample as normal
  - Air dries card and sends to the laboratory
- Parents are free to select whether or not the card is available for research
  - Important this does not deter parents from screening

Newborn Screening Consent

I have received and understood the information in the newborn screening brochure. I consent to my baby having blood collected for the newborn screening test.

☐ Yes  ☐ No

Secondary Research Use

I understand that blood from stored screening cards can be used occasionally for de-identified health research. I choose to make my baby’s blood sample available for this purpose.

☐ Yes  ☐ No

Parent Signature: ____________________________

When the blood sample is dry, mail this card without delay to:
Newborn Screening Laboratory
PO Box 1100, Parkville 3052
Ph: 8341 6272  Fax: 8341 6339  E: screeninglab@ghsv.org.au
Guthrie card sent to lab

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Parent Signature: ____________________________

When the blood sample is dry, mail this card without delay to:

Newborn Screening Laboratory
PO Box 1100, Parkville 3052
Ph: 8341 6272  Fax: 8341 6339  E: screeninglab@ghsv.org.au
Decline of screening possible

- Informed refusal is just as important as informed consent
  - If parents do not wish to screen
    - Ensure they have not been misinformed
    - Tick the NO box for screening and sign the card
    - Ensure front of card is completed and send to lab as normal
    - Complete a ‘decline’ form – this will be the hospital record
• In pilot
  – 0.2% refuse to participate in screening (used to be 0.04%)  
  – approx 90% support research use  

– Roll out to 55 public and 19 private maternity services in Vic by end of 2012, starting Aug 2011
Questions to consider?

– what is/should be the goal of NBS

– what should we screen for
  • who decides and how
    – microarrays / DNA chips / multiplex platforms
    – wider range of conditions
    – screen because we can (technological imperative) or ‘screen unless there is a compelling reason not to’

– what is acceptable
  • ethically
    – expand the notion of ‘benefit’
      » Remove diagnostic odyssey
      » Reproductive risk information

  • public resources – who pays?
Examples of possible NBS inclusions

- Alpha1 antitrypsin deficiency
- Fragile X
- Duchenne muscular dystrophy
Alpha 1 antitrypsin deficiency

Emphysema in young adults exacerbated by smoking
1 in 5000 US Caucasian and 1 in 500 European
• Added to NBS in Sweden
• Now 25 years later follow up has shown that carrier teens less likely to smoke

Maybe wait till 11-12 yrs when child is competent enough to understand and take part in screening decision
Both can go undiagnosed for many years (‘diagnostic odyssey’) – early recognition and intervention may:

- improve outcome for child
- allow for reproductive choice in subsequent pregnancies i.e. ‘reproductive benefit’

An alternative approach may be infant screening at say 6/12
3. Screening for chromosomal abnormalities in presence of developmental delay or multiple congenital abnormalities

Microarray-based comparative genomic hybridization (array CGH) is a technique to scan the genome for gains and losses of chromosomal material:

- submicroscopic deletions and duplications
- copy number variations, CNVs, can be caused by genomic rearrangements such as deletions, duplications, inversions, and translocations.
Molecular karyotyping - microarrays

- Compares DNA content of two differently labelled genomes - patient and control

- DNA from a test sample and normal reference sample are labelled differentially, using different fluorophores, and hybridized to a slide containing several thousand DNA probes (microarrays). The probes are derived from most of the known genes and non-coding regions of the genome, printed on a glass slide.
What is a microarray - up to $10^6$ ‘bits’ of information!!
An ~11kb deletion on chromosome 8 revealed by ultra-high resolution CGH. Blue lines: individuals with two copies. Red line: individual with zero copies.
Lab on a chip

Many different platforms
Positives

- Diagnostic yield varies from 5-20%

- Clinical utility inferred by
  - value of knowledge to the family
  - estimation of recurrence risk
  - importance of early detection and intervention
  - prognosis and future care
  - avoid further tests

- Expense somewhat offset by new Medicare rebate
  ($505; Medicare $307)
Negatives

• Often need to check parents to assess significance of CNV – can detect non-paternity
• Pathogenicity of alterations not always known – public databases required
• Only one of many possible related causes
• Regulatory and quality issues to be resolved – need standard guidelines
• Different labs use different platforms – confusing for clinicians

See Miller et al – Consensus statement 2010
The future is in genome sequencing
Next generation sequencing of the whole exome or whole genome and **targeted analysis** to look for:

- Single gene conditions
  - Predictive of later onset conditions
  - For diagnostic purposes (in affected individuals)
  - Carrier status (for recessive, X-linked single gene conditions)

??? Preconception carrier screening — reproductive risk
We all carry 2.8 disease mutations causing severe childhood disease.

Existing model (age) for Ashkenazi Jewish population

Maybe in IVF population; general population?
AND/OR

Extended analysis, genomic profiling, to look at whole genome sequence:

- $1000 genome by 2014
  - In clinical setting
  - Direct to Consumer

- Bioinformatics $$$$$

DNA personal health profiles

Children by design
Summary

- Traditional, established genetic testing programs exist for fetuses (karyotyping) and newborns (single gene tests)
- Non-invasive prenatal diagnosis and addition of further tests to newborn testing on the horizon
- Issue of informed consent being addressed in newborn testing setting
- New genetic testing programs introduced recently for children with dev delay and MCA (molecular karyotyping)
- Genome sequencing will become the ‘norm’ ??