Oncology and Genetics
Who, What and Why
Dr Hilda High
Genetic Oncologist
Why think about Genetics in Radiation Oncology?

• To pass exam
• To provide best patient care
• Because you’re a doctor and it’s in the papers and everyone expects you to know what it means
• So you don’t look like an idiot in MDT’s
• It’s fascinating !!
• Because there’s an attendance sheet

Outline

When / how to refer to Familial Cancer Services
Human genome and how we study it
How genes go wrong
Cancer syndromes and genetics
Radiosensitivity syndromes

(Please use references / resources for more information)
Why a Familial Cancer Service?

Risk prediction
Surveillance
Risk reduction strategies
Different treatment options
  Choosing mastectomy to avoid radiation
Patient may be at risk of other cancers
Other family members may be at risk
Reproductive choices

When to refer

3:2:1 = 3 blood relatives, 2 generations, 1 <50yrs

Patient characteristics
- Cancer at young age
- Multiple cancers in patient or family
- Syndromal features or cancer clustering
- Ethnicity re founder mutations

Tumour characteristics
- Pathology:
  - loss of staining for MMR proteins on IHC
- Rare tumour types
- Bilateral or multifocal tumours

eviQ  
Cancer Institute NSW  
• point of care  
• clinical information resource  
• current evidence based  
• peer maintained  
• best practice cancer treatment  
www.eviQ.org.au
Sydney Cancer Genetics is a specialised medical service supporting individuals and families concerned about cancer.

Our services include:
- assessment of inherited risk
- genetic counselling and testing
- cancer prevention information
- cancer risk management
- telehealth to regional and rural areas

Our staff include:
Dr Hilda High, a Genetic Oncologist, who works at the Northern Haematology & Oncology Group in the SAN clinic in Wahroonga.
Family History

Daughter attends with mum

27

Mother
Br ca 58

Daughter attends with mum
27
Importance of Family History

Breast ca 68

Breast ca 60

Cervical / uterine ca 45

Mother
Breast ca 58

Daughter attends with mum

27
Importance of confirming pathology

Daughter attends with mum

Breast ca 60

Bilateral breast ca age 34

Breast ca 68

Ovarian ca 48

Mother Breast ca 58

Daughter attends with mum 27

58
What Family Cancer Services Do

Verification
  - cancer family history (most conditions autosomal dominant)

Genetic counselling +/- testing
  - Probability of germline mutation
  - Pre test counselling
    - DNA (blood) testing of affected individual (= proband)

Quantify cancer risk and provide advice
  - Especially when testing is inconclusive (ie: no mutation found on mutation search in proband)
    - early detection, risk reduction, offspring

Multidisciplinary cancer care

Why Cancer Runs in Families?

Single Gene
Gene/gene interactions
Gene/environment interactions
Chance
Diet / lifestyle
A few terms

Autosome
non sex chromosome

Autosomal dominant
need only one faulty gene, from mum or from dad

Autosomal recessive
need a faulty gene from each parent

Imprinting  (epigenetics)
the “parent of origin” makes a difference to whether the faulty gene causes problems

Note: faulty genes don’t skip generations but the cancers may
And a few more terms

• Carrier frequency
  – How common a mutated gene is within the population
  – eg Cystic Fibrosis and Haemochromatosis

• Founder effect / Founder mutation
  – A population that is isolated (culturally or geographically) may develop a high frequency of a mutated gene
  – eg 3 Ashkenazi BRCA mutations

Founder Effect

eg: BRCA 1 and BRCA 2
Frequency in general population = 1:1000
Frequency in Ashkenazi Jewish Population = 1:50

“Bottleneck”
Population reduced in size

Population re-expands but remains culturally or geographically isolated
And... a few more terms

Genotype vs phenotype

Different syndrome (phenotype) depending on where the mutation is (genotype). Eg MEN2

Penetrance

Few cancer syndromes have 100% likelihood of cancer.

FAP = 100%, Lynch = 30 to 40%

Phenocopies

Cancer is common – 10% of women get breast cancer

Genetic heterogeneity

4 mismatch repair genes – Lynch syndrome

Somatic vs Germline Mutations

**Somatic:** occurred locally in an individual cell (e.g., breast or bowel)

  Random / spontaneous. Not inherited. This is most cancer

**Germline:** From germ cells (egg / sperm). Mutation in every cell

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[Diagram showing somatic and germline mutations]

Key:
- **Body cells containing faulty (mutated) gene**
- **Body cell containing working copy of gene**

[Diagram showing inheritance of a mutation in a germ cell]

Key:
- **Body cells containing faulty (mutated) gene**
- **Faulty (mutated) control gene in all cells**

1839 Cell Theory
1859 Theory of Evolution
1882 Chromosomes observed
1903 Chromosomes carry genes
1944 Role of DNA
1953 DNA structure
1956 46 Chromosomes in humans
1970 Chromosomal banding
1975 DNA sequencing
1979 In vitro fertilisation
1986 Polymerase Chain Reaction (PCR)
1987 Linkage map of human chromosomes developed
1993 First physical map of human genome
2000 First draft of human genome
2003 Completion of human genome sequence
2008 International 1000 genomes project launched

1960 Philadelphia chromosome
1971 Knutson 2 Hit hypothesis
1999 Cancer Genome Project
2002 miRNA and cancer
Human Genome Project

Started in 1990
Aim: to identify and sequence all genes (and make publically available)
Needed sequencing, computer analysis
Hierarchial shotgun (location known) vs whole genome shotgun (relied on overlap)
Both used Sanger sequencing
Available via genome browser eg Ensembl
Genetic Errors

DNA code made up of A, T, G, C
3 nucleotides code for an amino acid (aa)
(or an order such as a “stop codon”)
More than one way to code for an amino acid
Changing one nucleotide (point mutation) may
Not change the aa = silent or synonymous
May change aa = missense or non synonymous
  If is a similar aa = neutral
  or may be different and disrupt the protein
May be stop codon = truncating / nonsense
If insert / delete and not divisible by 3 = Frame shift
Polymorphism = common change of no effect

## Types of Genetic Errors

<table>
<thead>
<tr>
<th>Types of Errors</th>
<th>Detection Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point mutations</td>
<td>Sequence</td>
</tr>
<tr>
<td>Insertions / deletions (Indels)</td>
<td>Sequence</td>
</tr>
<tr>
<td>Large deletions</td>
<td>MLPA</td>
</tr>
<tr>
<td>Copy number variants CNV</td>
<td>aCGH / FISH</td>
</tr>
<tr>
<td>Translocations and Rearrangements</td>
<td>aCGH / FISH</td>
</tr>
<tr>
<td>Chromosomal</td>
<td>karyotype</td>
</tr>
<tr>
<td>Infections eg HPV in tumour</td>
<td></td>
</tr>
</tbody>
</table>
Chromosomal Change

Germline:
- Down Syndrome
- Trisomy 21
- Increased risk leukaemia

Somatic:
- Philadelphia Chromosome
  - Translocation: BRC-ABL
- EML4-ALK & lung cancer
Polymerase Chain Reaction

DNA
Two primers that are complementary to the DNA (sense and antisense)
DNA polymerase
Nucleotides (dNTPs)
Heat to denature DNA (single strand), cool to anneal primers, warm to added nucleotides
Repeat to get lots of short lengths of DNA
Genetic testing - Sanger Sequencing

Template DNA (from Human Genome Project)
PCR using dNTP and ddNTPs
Terminate when ddNTPs added
Each fluorescently labelled
Computer reads and shows nucleotide sequence
    Forward and reverse to make sure is “real”
Sanger Sequence of part of BRCA2

Electropherogram of BRCA 2 variant

Reference (wt)
c.506A>G
Gene Amplification

FISH or array
Frequently used in the tumour
  eg HER2 over-expression in breast cancer

Also used in germline eg Trisomy 21 (Down syndrome)
MLPA

How do you find something that is missing?

1. Universal Primer
   Target Specific sequence ~20bp
   Stuffer Sequence ~100-400bp
   MLPA Probes with “stuffer” sequence.

2. MLPA Probes bind to consecutive nucleotides on target DNA
   Target Sequence

3. Probes are ligated then amplified via PCR
   Ligation

4. Amplified probes can now be separated based on size. ~150 – 450bp
   PCR Product
Array Comparative Genomic Hybridisation (aCGH)

Fluorescence measured for each spot (can you see the red one?)

Plotted on chromosome map to reveal copy number variations

Array CGH slide
Next Generation Sequencing
aka Massively Parallel sequencing
Reagents cheaper and is faster
BUT Terabytes of data that needs to be interpreted
Either one person thousands of genes
  whole exome sequencing (pt and tumour)
or thousand of people for a few genes
  panel testing (multiple breast / bowel cancer genes)
Finding Mutations is the Easy Part!

NCBI SNP database
In silico – guessing from experience
What amino acid substitution means:

SIFT – Sorting Intolerant From Tolerant
Polyphen – prediction of functional effect

Conservation across species
<table>
<thead>
<tr>
<th>Organ or neoplasia type</th>
<th>Histologic type</th>
<th>Genetic differential diagnosis</th>
<th>Gene (if known)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowden syndrome/</td>
<td>Papillary thyroid carcinoma</td>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td>Bannayan–Riley–Ribsalva syndrome</td>
<td></td>
<td>TP53</td>
<td></td>
</tr>
<tr>
<td>Li–Fraumeni syndrome</td>
<td>Osteosarcoma</td>
<td>BRCA1</td>
<td></td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome</td>
<td>Intestinal polyposis</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>Axiatal angiectasia</td>
<td>Gastrointestinal stromal tumor</td>
<td>KIT</td>
<td></td>
</tr>
<tr>
<td>Hereditary breast-ovarian cancer syndrome</td>
<td>Breast,ovarian carcinoma</td>
<td>BRCA2</td>
<td></td>
</tr>
<tr>
<td>Cowden syndrome</td>
<td>Papillary thyroid carcinoma</td>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td>Retinoblastoma syndrome</td>
<td>Retinoblastoma</td>
<td>RB1</td>
<td></td>
</tr>
<tr>
<td>Klinefelter syndrome</td>
<td>Male gonadal dysgenesis</td>
<td>XXY</td>
<td></td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Adenocarcinoma</td>
<td>Hereditary non-polyposis colorectal cancer syndrome</td>
<td>MLH1, MSH2, MSH6, PMS1, PMS2, APC</td>
</tr>
<tr>
<td>Familial adenomatous polyposis syndrome</td>
<td>Familial adenomatous polyposis adenoma</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>Juvenile polyposis syndrome</td>
<td>Juvenile polyposis syndrome</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>SMAD4</td>
<td>SMAD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMPRIA</td>
<td>BMPRIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome</td>
<td>Peutz–Jeghers syndrome</td>
<td>BMPRIA</td>
<td></td>
</tr>
<tr>
<td>Hereditary mixed polyposis syndrome(s)</td>
<td>Hereditary mixed polyposis syndrome(s)</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>MAP2K1</td>
<td>MAP2K1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUTYH-associated polyposis</td>
<td>MUTYH-associated polyposis</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>Birt–Hogg–Dubé syndrome</td>
<td>Birt–Hogg–Dubé syndrome</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>Hereditary non-polyposis colorectal cancer syndrome</td>
<td>Hereditary non-polyposis colorectal cancer syndrome</td>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>Familial adenomatous polyposis syndrome</td>
<td>Familial adenomatous polyposis syndrome</td>
<td>APC</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
Retinoblastoma

If bilateral or family history, the likelihood of a germline mutation is:

- 10%
- 20%
- 50%
- ~100%
Retinoblastoma

Rb: Tumour suppressor gene

Eye tumour - Most <5yrs
60% unilateral; 40% bilateral

If bilateral or family history ~100% will have germline
15% if unilateral and no family history

Test tumour:
idea is to identify 2 mutations and then exclude in germline

Screening:
Eye exam every 3 to 4 weeks for 1st year then less frequently until 3yrs
Associated increased risk sarcoma – no specific screening

Avoid DNA damaging agents: radiotherapy, tobacco, and UV light

Knudson 2 hit hypothesis

Chromosome 13 pair in the normal cells of an individual with a predisposition to retinoblastoma

Non-disjunction (loss)  Non-disjunction and reduplication  Mitotic recombination  Gene conversion  Deletion  Point mutation

Mechanism 1  Mechanism 2  Mechanism 3  Mechanism 4  Mechanism 5  Mechanism 6

Six possible chromosome 13 pairs that might be seen in a retinoblastoma tumour arising in the individual whose (non-tumour cell) chromosomes are shown at the top of the figure.
Li Fraumeni syndrome

Mutation in TP53 (protein = p53)
Multiple cancers, young age
  Sarcoma
  Lung, leukaemia
  Breast, Brain

Chompret Criteria

TP53 commonly mutated in sporadic cancers
LiFraumeni Cancer Risks
Which is false

1. Risk of cancer is 15% by age 30
2. No screening except for breast in women
3. Breast cancers likely to be triple positive
4. Breast cancer screening, including MRI, starts at 20yrs
Li Fraumeni syndrome
Breast: 4.8% of breast cancer <30
especially if triple positive: ER+/PR+/HER2+
Risk Reducing Mastectomy or MRI from age 20
No evidence for screening for other cancers
Avoid smoking, UV and radiation

<table>
<thead>
<tr>
<th>Lifetime Cancer risks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
</tr>
<tr>
<td>15% by 20 yrs</td>
</tr>
<tr>
<td>50% by 30 yrs</td>
</tr>
<tr>
<td>&gt;90% by 50 yrs</td>
</tr>
<tr>
<td><strong>Male</strong></td>
</tr>
<tr>
<td>15% by 20 yrs</td>
</tr>
<tr>
<td>20% by 30 yrs</td>
</tr>
<tr>
<td>60% by 50 yrs</td>
</tr>
</tbody>
</table>

Lynch Syndrome

• Caused by a mutation in one of the mismatch repair genes MLH1, MSH2, MSH6 or PMS2.
• produce proteins = “spell checkers”
• In tumour, both copies damaged = loss of protein
  – = loss of immunohistochemical (IHC) staining (MMR IHC)
• Proteins work in pairs
  – MLH1 with PMS2 and MSH2 with MSH6
  – if the dominant protein is missing, it’s partner missing too
If the gene isn’t working the protein isn’t made. Staining will be “negative”

Slides provided by the pathology department at
The Sydney Adventist Hospital, Wahroonga, Sydney

Lynch Syndrome

- Accounts for 3% bowel and 3% uterine (1:35)
- Penetrance less than previously thought:

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Lynch (70 yrs)</th>
<th>General Population (85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon; Male</td>
<td>40%</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>Colon; female</td>
<td>35%</td>
<td>5 to 6%</td>
</tr>
<tr>
<td>Uterine</td>
<td>35%</td>
<td>2 to 3%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>10%</td>
<td>1 to 2%</td>
</tr>
</tbody>
</table>

Thus testing based on FHx identifies <25%!

Universal testing of all colon cancers and ? uterine and others
Other family members at risk; **patient at risk of second cancer**
Screening effective

Who should see a Genetic Oncologist

Bowel cancer < 50 years or with loss of staining

Polyps
- Young age (eg 3 by age 30)
- Lots of polyps (eg >20 over time)
- 3 or more “special” polyps
  - hamartomatous polyps
  - juvenile polyps at any age

Woman with uterine cancer <50

Family history
- 2 bowel cancers, where one occurred under age 50
- 3 cancers belonging to Lynch syndrome (bowel, uterine, ovarian etc)
BRCA1 and BRCA2

Autosomal dominant
   (ie: 50% chance of inheriting from mother or father)
High penetrance
breast and ovarian cancer
   BRCA1 often triple negative
BRCA2 also prostate, pancreas, melanoma

Lifetime Risks

<table>
<thead>
<tr>
<th>Risk</th>
<th>Breast</th>
<th>Av age</th>
<th>Ovarian</th>
<th>Av age</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Population</td>
<td>1 in 11 by age 70</td>
<td>&gt; 60</td>
<td>1 in 100 by age 70</td>
<td>&gt; 63</td>
</tr>
<tr>
<td></td>
<td>8% lifetime</td>
<td></td>
<td>1% lifetime</td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>40 to 80% lifetime</td>
<td>50</td>
<td>40 to 60% lifetime</td>
<td>50 to 55</td>
</tr>
<tr>
<td>BRCA2</td>
<td>40 to 80% lifetime</td>
<td>55 to 57</td>
<td>10 to 20% lifetime</td>
<td>60 to 65</td>
</tr>
</tbody>
</table>
Risk of Contralateral Breast Cancer in BRCA1 mutation carrier <40 at diagnosis of first breast cancer

1. 10%
2. 20%
3. 40%
4. 60%
5. >60%
Managing Cancer Risk in BRCA+

Breast:
- Risk Reducing Surgery
- Contralateral breast cancer risk:
  - BRCA1: $63\%$ if pt $<40$ and $20\%$ if pt $>50$ at diagnosis
- Screening: Starting from age 30; Including MRI to age 50
- Risk Reducing Medication eg Tamoxifen

Ovarian:
- Risk Reducing Surgery
- Screening with transvaginal US and Ca125 doesn’t work

Don’t forget the non-personalised (public health):
- Diet / exercise / healthy body weight / lifestyle

Tubal Origin of Ovarian Ca in BRCA+

Early dissemination is the hallmark of BRCA related high grade serous cancer: Time to accept the futility of screening and the importance of appropriate risk reducing surgery in mutation carriers. HA High and M Friedlander. kConFab 2011.
Benefit of RRSO

If around 40 / before menopause:
- 50% reduction in breast cancer risk
- 98% reduction in ovarian cancer risk

Domchek PROSE study 2010
- all-cause mortality: 10% vs 3%
  $HR = 0.40$ [95% CI, 0.26-0.61]

HRT can be used to age of natural menopause

Germline vs Epigentic

If loss of staining for MLH1 (common in older pts)
   test BRAF (BRAF V600E)
   If BRAF mutated, means somatic change
      not Lynch, not heritable

Now know can have promotor hypermethylation which silences the gene
   Loss of staining but no mutation on germline testing
Can even be inherited (0.6% of MLH1)
Epigenetic change

PWS = Prader Willi syndrome   AS = Angelman syndrome  Black circle = CpG methylation

Radiosensitivity Syndromes

Mechanism

Impaired DNA repair / chromosomal breakage
  Ataxia Telangiectasia (AT)

Impaired cell cycle check point functioning
  Li Fraumeni (TP53)

Tests (fibroblasts or lymphocytes)

Cell survival

Micronucleous

G2 chromosomal assay

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gene</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td>ATM</td>
<td>AR homo + heterozygous</td>
</tr>
<tr>
<td><strong>Gorlin syndrome</strong> <em>(Basal cell nevus syndrome)</em></td>
<td>PTCH1</td>
<td>AD</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td>BLM</td>
<td>AR</td>
</tr>
<tr>
<td>Common variable immune disorder</td>
<td>various</td>
<td></td>
</tr>
<tr>
<td>Down syndrome <em>(trisomy 21)</em></td>
<td>various</td>
<td></td>
</tr>
<tr>
<td>Dyskeratosis congenita</td>
<td>various</td>
<td>AR, x-linked, AD</td>
</tr>
<tr>
<td>Familial dysplastic naevus syndrome</td>
<td>CDKN2A</td>
<td></td>
</tr>
<tr>
<td>Fanconi anaemia</td>
<td>various</td>
<td>AR</td>
</tr>
<tr>
<td>Gardner syndrome <em>(Familial Adenomatous Polyposis)</em></td>
<td>APC</td>
<td>AD</td>
</tr>
<tr>
<td>Klinefelter syndrome</td>
<td>XXY</td>
<td>chromosomal</td>
</tr>
<tr>
<td>Li Fraumeni syndrome</td>
<td>TP53</td>
<td>AD</td>
</tr>
<tr>
<td>Nijmegen breakage syndrome</td>
<td>NBS1</td>
<td>AR</td>
</tr>
<tr>
<td>Neurofibromatosis type 1 or type 2</td>
<td>NF1, NF2</td>
<td>AD</td>
</tr>
<tr>
<td>Rothmund Thomson syndrome</td>
<td>RECQL4</td>
<td>AR</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>RB</td>
<td>AD</td>
</tr>
<tr>
<td>Wilms tumour</td>
<td>11p del</td>
<td>AD</td>
</tr>
<tr>
<td>Xeroderma pigmentosa</td>
<td>various</td>
<td>AR</td>
</tr>
</tbody>
</table>
References


Esssential Medical Genetics by Tobias ES, Connor M, Ferguson-Smith M. Wiley and Blackwell (downloadable version – many figures in this presentation from this reference)


Centre for genetics education – www.genetics.edu.au
