PROGRAMME

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Tuesday 21st August – Olympic Room

8.30 – 8.40 Welcome: Graeme Suthers

FCC Session 1 - Olympic Room

Rare presentations of HNPCC
Chairperson: Lara Lipton

8.40 – 9.00 Gynecological Aspects of HNPCC
David Allen

9.00 – 9.20 Familial Cancer: Lessons from the Skin
Ingrid Winship

9.20 – 9.40 Clinical Spectrum of Biallelic HNPCC Mutations
Tiong Tan

9.40 – 10.30 Case Study Discussion Panel with Guest Presenters and Yoland Antill

10.30 –11.00 Morning tea

FCC Session 2 – Olympic Room

Clinical Aspects of Hyperplastic Polyposis
Chairperson: Elizabeth Chow

11.00 – 11.20 Phenotypic Characteristics of Hyperplastic Polyposis Syndrome
Elizabeth Chow

11.20 – 11.40 Pathology of Hyperplastic Polyposis
Chris Dow

11.40 – 12.00 Hyperplastic Polyposis and the Genetics of Serrated Neoplasia
Joanne Young

12.00 – 12.30 Questions and comments from the panel.
12.30 – 1.30  Lunch

FCC Session 3 – Olympic Room
*Phaeochromocytoma Syndrome*
Chairperson: Mike Field

1.30 – 2.15  *Towards an Understanding of Familial Phaeochromocytoma and Paraganglioma.*
Dindy Benn

2.15 – 2.30  *Inherited Paraganglioma Syndromes at Prince Of Wales Hospital Randwick and Outreach Services*
Kathy Tucker

2.30 – 2.45  *Familial Paraganglioma: Challenges in The Genetic Counselling Setting*
Maira Kentwell

2.45 – 3.15  Panel Discussion with guest presenters and *Gareth Evans*

3.15 – 3.45  Afternoon tea

FCC Session 4 – Olympic Room
*Studies of Familial Renal Cancer*
Chairperson: Ingrid Winship

3.45 – 4.15  *Study Update*
Tracy Dudding

FCC Session 5 – Olympic Room
*Notifying Relatives without Consent*
Chairperson: Gerda Evans

4.15 – 4.30  *Challenging Counselling Issues in a Family with Hereditary Diffuse Gastric Cancer*
Georgina Fenton

4.30 – 4.45  *Letting the Family Know - Without Consent*
Graeme Suthers

4.45 – 5.15  Questions and discussion

FCC Staff - Drinks and Dinner at the Pool-side café (paid for by the delegates)
Wednesday 22nd August  
kConFab and AOCS

Session 1 – Olympic Room

kConFab, AOCS and National Updates  
Chairperson: Judy Kirk

8.30 – 8.50  
The Annual kConFab Update of Core Activities  
Georgia Chenevix-Trench

8.50 – 9.10  
kConFab Clinical Follow Up Study  
Kelly Phillips

9.10 – 9.30  
The Australian Ovarian Cancer Study update  
Anna DeFazio

9.30 – 10.00  
Donor Perspectives with Regard to Donation of Samples and Information: A Legal Overview of Key Issues  
Margaret Otlowski

10.00 – 10.10  
PARPi and IMPACT Trial Update  
Gillian Mitchell

10.10 - 10.20  
National MRI Update  
Christobel Saunders

10.30 – 11.00  
Morning tea

Session 2a – Olympic Room

Molecular Pathology  
Chairperson: Stephen Fox

11.00 – 11.40  
Molecular Classification Of Breast Cancer: HER2 and Basal-Like Phenotypes, Two Aggressive Classes, Different Genes Involved but Common Metastatic Patterns  
Frederique Llorca

11.40 – 12.00  
Aberrant Expression of E-Cadherin in Lobular Carcinoma of the Breast: A Morphological And Molecular Analysis of a Consecutive Series of Cases  
Leonard Da Silva

12.00 – 12.20  
Genetic Alteration Is a Rare Event in Breast And Ovarian Carcinoma Associated with Stroma  
Ian Campbell

12.20 – 12.40  
Loss of Heterozygosity at the BRCA2 Locus Is a Common Finding in Prostate Cancer in Men with a Pathogenic Germline Mutation  
Amber Willems
12.40 – 1.00  Sebaceous Adenoma As a Marker of HNPCC/Lynch Syndrome  
Graeme Suthers

11.00 – 1.00  Session 2b – Mango Room  
kConfab Research Nurse Annual Meeting

1.00 - 2.00  Lunch

Session 3 –  Olympic Room  
Phenocopies in BRCA1/2 Breast Cancer Families  
Chairperson: Graeme Suthers

2.00 – 2.30  Phenocopies in BRCA1 and BRCA2 Families: Evidence for Modifier Genes or Biased Ascertainment?  
Gareth Evans

2.30 – 2.40  Phenocopies in the Kathleen Cuningham Consortium for Research on Familial Breast Cancer (kConFab) Cohort  
Judy Kirk

2.40 – 2.50  Breast Cancer Phenocopies in BRCA-Families in the Family Cancer Clinics  
Nicola Poplawski

2.50 – 3.00  The Epidemiologist’s Point of View  
David Goldgar

3.00 – 3.30  Discussion

3.30 – 4.00  Afternoon Tea

Session 4 –  Olympic Room  
Breast Cancer Profiling  
Chairperson: Ian Campbell

4.00 – 4.40  Molecular Strategies Improve Therapeutic Decisions  
Laura Van’t Veer

4.40 – 5.00  Expression Profiling Reveals Sub Groups within Familial Breast Tumours  
Nic Waddell

5.00 – 5.20  Clinical Classification of BRCA1 and BRCA2 Missense Substitutions – Potential Low-Moderate Risk Variants Identified by Multifactorial Analysis, Functional Studies, and Germline Microarray Profiling.  
Amanda Spurdle
5.20 – 5.40  Identification of Novel Mutation Targets in The Breast Cancer Susceptibility Gene BRCA1 And Genes That Encode Regulators Of BRCA1
Melissa Brown

Delegates Organise their own Dinner

7.30 - 10.30 Poster Session + Wine and Cheese in the Pavilion, Blue Gum Point
Sponsored by: Millennium Sciences with a Student Prize to be Awarded
<table>
<thead>
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<th>Time</th>
<th>Event</th>
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| 8.30 - 8.50 | **kConFab Annual General Meeting**  
Olympic Room |
| Session 5 | **Olympic Room**  
National Updates  
Chairperson: Graham Mann |
| 8.55 – 9.05 | Amendment to the Federal Privacy Act – Notifying Relatives  
Graeme Suthers |
| 9.05 – 9.15 | Family Cancer Information Link – Breast Cancer Network  
Gerda Evans |
| Session 6a – | Jackson Landy Room  
Attitudes Towards Cancer Prevention Strategies  
Chairperson: Bettina Meiser |
| 9.15 – 9.50 | Preventive strategies for HBOC: Contextual Interpretation of International Discrepancies in Attitudes and Behaviours  
Clair Julian-Reynier |
| 9.50 – 10.05 | Barriers to the Discussion of Breast Cancer Chemoprevention in Australian Family Cancer Clinics  
Louise Keogh |
| 10.05 – 10.20 | Attitudes to Use of Surgical Removed Tissue For Research  
Lesley Andrews |
| Session 6b – | Olympic Room  
DNA Repair and Cancer  
Chairperson: Melissa Brown |
| 9.15 – 9.50 | DNA Damage Induced by Chronic Inflammation is a Major Contributor To Carcinogenesis  
Leona Samson |
| 9.50 – 10.05 | PMS2 Mutations and Cancer: An Update of South Australia’s Experience  
Nicola Poplawski |
| 10.05 – 10.20 | DNA Mismatch Repair Deficiency in Early On Set Endometrial Carcinomas  
Michael Walsh |
| 10.20 – 10.35 | Do we need both MSI and IHC for triaging HNPCC  
Barney Rudzki |
10.35 - 11.00  Morning tea

Session 7 –  Olympic Room
Ovarian Cancer
Chairperson: Anna DeFazio

11.00 – 11.40  Pathology of Familial Ovarian Carcinoma
Blake Gilks
Supported by VPGH Ltd

11.40 – 12.00  Molecular Sub-Classification of Ovarian Cancer by Expression Profiling
Richard Tothill

12.00 – 12.20  Biomarker and Functional Roles of the Serine Protease KLK7 and Its Novel Splice Site Variant in Epithelial Ovarian Cancer
Olivia Tan

12.20 – 12.40  Collagen and Calcium Binding EGF Domains 1 (CCBE1): A Novel Tumour Suppressor Gene in Ovarian Cancer
Caroline Barton

12.40 – 1.00  Characterisation of MicroRNA Genes in Ovarian Cancer
Jennifer Bearfoot

1.00 - 2.00  Lunch

Session 8a –  Olympic Room
Genetic Epidemiology
Chairperson: John Hopper

2.00 – 2.40  A Genome-Wide Association Study of Breast Cancer
David Hunter

2.40 – 3.00  Do Breast Cancer Predisposition Genes also Predispose to Prostate Cancer?
Fabrice Odefrey

3.00 – 3.20  MDR-1 Single Nucleotide Polymorphisms and Ovarian Cancer Relapse And Survival
Sharon Johnatty

3.20 – 3.40  Talcum Powder, Chronic Pelvic Inflammation and Nsaids in Relation To Risk Of Epithelial Ovarian Cancer
Penny Webb
Session 8b – Landy Jackson Room

**Psychosocial Impact of Genetic Testing**

Chairperson: Melanie Price

2.00 – 2.40  Cognitive and Behavioral Adjustments Two Years after An Inconclusive Genetic Test Result In a Cohort Of HBOC Affected Women

  Clair Julian -Reynier


  Liz Lobb

3.00 – 3.20  Familial Cancer Centres Emerging Lifetime Role for Individuals with Mutations in Cancer Predisposition Genes

  Lucinda Hossack

3.20 – 3.40  A Randomised Controlled Trial of a Decision Aid for Individuals Considering Genetic Testing for Hereditary Non Polyposis Colorectal Cancer (HNPCC)

  Bettina Meiser

3.40 – 4.00  Afternoon tea

4.00 – 5.30  Free Time

5.30 – 7.00  Cocktails @ the Surf Club for all

  (Free bus trolleys to and from the Surf Club)

7.30 – 11.30  Conference Dinner @ the Surf Club
Friday 24th August kConFab and AOCS

Session 9 – Olympic Room
Modifiers and Moderate Risk Genes
Chairperson: Georgia Chenevix-Trench

Antonis Antoniou

9.50 – 10.10 Variants Of ATM, PALB2 and CHEK2 May Have High Penetrance for Most Women with a Strong Family History
John Hopper

Session 10 – Olympic Room
Risk Prediction Models
Chairperson: Gillian Mitchell

10.10 – 10.30 Risk Prediction – Introduction and The Logistic Regression Model
David Goldgar

10.30 – 11.00 Morning tea

Session 10 – Olympic Room
Risk Prediction Models (continued)
Chairperson: Gillian Mitchell

11.00 – 11.15 Boadicea and kConFab
Antonis Antoniou

11.15 – 11.30 Tyler Cuzick and kConFab
Graham Mann

11.30 – 11.45 BRCAPRO and kConFab
James Dowty

11.00 – 11.15 Goodbye Adelaide and Hello Manchester
Graeme Suthers

11.15 – 11.30 The Manchester Scoring System for BRCA1/2 Mutations: An Update
Gareth Evans

12.30 – 12.40 Summary
David Goldgar

12.40 – 1.00 Discussion
Tuesday 21 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand.

“Familial Cancer 2007: Research and Practice”

FCC Session 1 – Olympic Room
Rare Presentations of HNPCC

Chairperson: Lara Lipton

“Familial Cancer 2007: Research and Practice”
GYNAECOLOGICAL ASPECTS OF HNPCC

David Allen
Mercy Hospital for Women, University of Melbourne

HNPCC is caused by mutations of DNA MMR genes and is estimated to occur at a population frequency of 1/1000-2000. The transmission is autosomal dominant (50% affected). The clinical criteria Amsterdam II.

Gynaecological cancers include endometrial cancer (about 40%) and ovarian cancer (about 20%).

Annual surveillance remains controversial. Gynaecological examination is generally not helpful. TVUS and CA 125 are of limited benefit. Reporting symptoms remains important.

Germline mutations in the MMR-genes cause 2% of ovarian cancers

The position statement of The Cancer Council Victoria on Gynaecological Cancer Surveillance Screening for HNPCC will be presented.

The option of prophylactic hysterectomy will be discussed.

References:


Malander S, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. Gynecol Oncol. 2006 May;101(2):238-43. (Lund University Hospital, Sweden).


FAMILIAL CANCER: LESSONS FROM THE SKIN

Ingrid Winship, Royal Melbourne Hospital, Melbourne

As the molecular basis of disease continues to be elucidated, familial cancer syndromes, comprising a constellation of malignant and non-tumour features, are emerging. The usual pathway to a genetic clinic, or familial cancer centre, is via an oncologist when high risk features indicating a possible hereditary basis for the presenting cancer are recognised. Traditionally, these high risk features include multiple family members with similar cancers over two or more generations, young age of onset and multiple synchronous or metachronous tumours. These high risk features are effective in ascertaining families with hereditary breast and ovarian cancer due to a BRCA mutation, or Hereditary Non Polyposis Colon Cancer (HNPCC) and Familial Adenomatous Polyposis (FAP). However, there are familial cancer syndromes, which remain undiagnosed without extending the history and examination to include non-malignant features. The identification of cutaneous lesions associated with rare familial cancer syndromes provides individuals and their families the opportunity to undertake early surveillance for malignant and non-malignant complications. I will describe a series of patients where physical signs could have alerted health professionals to the more significant clinical features in Birt Hogg Dube Syndrome, HLRCC, Carney Syndrome, Cowden Syndrome and Multiple Endocrine Neoplasia. Early intervention through surveillance when offered to at risk families, may improve clinical outcomes.
BIALLELIC MUTATIONS IN MISMATCH REPAIR GENES

Tiong Yang Tan¹, Lisa Orme², Elly Lynch¹, Lara Lipton³

¹ Genetic Health Services Victoria, Murdoch Children’s Research Institute, Department of Paediatrics, University of Melbourne, Royal Children’s Hospital, Melbourne, Australia
² Children’s Cancer Centre, Royal Children’s Hospital, Melbourne, Australia
³ Familial Cancer Service, Royal Melbourne Hospital, Melbourne, Australia

Homozygous mutations in mismatch repair (MMR) genes are associated with a childhood cancer syndrome, distinct to Hereditary Non-Polyposis Colorectal Cancer (HNPCC) caused by heterozygous mutations in the same genes. The condition results in early onset of unusual childhood cancers and atypical café-au-lait macules. The tumour spectrum includes brain and haematological malignancies, colorectal polyposis and cancer. Onset is as young as 2 years. The risk of second malignancy is high. Tumour surveillance is of uncertain benefit and raises considerable anxiety. A consanguineous Lebanese-Australian family with at least two affected children (one demonstrated to have a homozygous PMS2 mutation) will be presented. The medical and psychosocial issues associated with biallelic mutations in MMR genes will be reviewed.
Tuesday 21 August

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“Familial Cancer 2007: Research and Practice”

FCC Session 2: Olympic Room
Clinical Aspects of Hyperplastic Polyposis

Chairperson: Elizabeth Chow
PHENOTYPIC CHARACTERISTICS OF HYPERPLASTIC POLYPOSIS SYNDROME
Elizabeth Chow

Hyperplastic Polyposis Syndrome (HPS) is a condition poorly classified by phenotype and genotype, comprising multiple, large and/or proximal hyperplastic polyps (HP). Relatively little is known about its aetiology and natural history nor its associated risk for cancer development. Recent reports suggest a link between HPS and microsatellite unstable colorectal cancer. Review of published case reports in the literature will be presented.

Presentation of 38 HPS patients phenotype
Utilising clinical databases of The Royal Melbourne Hospital Cancer Surveillance Service and the Familial Cancer Clinic, 38 patients with HPS have been recruited. All patients have multiple, large and/or right sided HPs. All patients have colonoscopic surveillance to follow the natural progression of their disease. The pedigrees have been verified, and where possible, multiple colonoscopy and histopathology findings as well as molecular genetic testing have been ascertained. The cohort of patients was analysed for age at first diagnosis, features of hyperplastic polyposis (number, site, size of largest HP and number of HPs larger than 10mm), characteristics of coexisting adenomas (number, location, dysplasia, largest adenoma size), presence of serrated adenomas, and incidence of colorectal cancer. Testing for microsatellite instability (MSI) in a subset of cancers and polyps was performed. Family histories of polyposis and colorectal cancers were carefully documented. 38 germline DNA samples and cell lines have been collected.

Among this series of 38 patients with HPS, serrated adenomas are common (26%). 50% of the 38 patients have a first degree relative with CRC. Family history of HPS is uncommon, only two cases of familial aggregation of HPS were described. 10 patients developed CRC at a young mean age of 49. 3 patients required total colectomy and ileo-rectal anastomosis for polyposis, all 10 patients with colorectal cancers required lesser colonic resections.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
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<tbody>
<tr>
<td>Female/Male</td>
<td>21/17</td>
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<tr>
<td>Mean age</td>
<td>57</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>52 (32-79)</td>
</tr>
<tr>
<td>Mean age at first diagnosis</td>
<td>49</td>
</tr>
<tr>
<td>Median (range) age at first diagnosis</td>
<td>44 (27-78)</td>
</tr>
<tr>
<td>Mean number of colonoscopies</td>
<td>5</td>
</tr>
<tr>
<td>Median number (range) of colonoscopies</td>
<td>5 (1-15)</td>
</tr>
<tr>
<td>Number of patients with HPs proximal to sigmoid</td>
<td>37 (97%)</td>
</tr>
<tr>
<td>Number of patients with HPs &gt;10mm</td>
<td>15 (39.5%)</td>
</tr>
<tr>
<td>Number of patients with more than 5 coexisting adenomas</td>
<td>12 (32%)</td>
</tr>
<tr>
<td>Number of patients with at least 1 serrated adenoma</td>
<td>10 (26%)</td>
</tr>
<tr>
<td>Number of patients with colorectal cancer</td>
<td>10</td>
</tr>
<tr>
<td>Mean age of diagnosis of colorectal cancer</td>
<td>49</td>
</tr>
<tr>
<td>Median (range) age of diagnosis of colorectal cancer</td>
<td>45 (31-71)</td>
</tr>
<tr>
<td>Number of patients with family history of colorectal cancer</td>
<td>24 (63%)</td>
</tr>
<tr>
<td>Number of patients with first degree relative with colorectal cancer</td>
<td>19 (50%)</td>
</tr>
<tr>
<td>Number of families with family history of hyperplastic polyposis</td>
<td>2</td>
</tr>
<tr>
<td>Number of patients receiving major colonic resection for polyposis</td>
<td>3</td>
</tr>
<tr>
<td>Number of patients receiving major colonic resection for cancer</td>
<td>10</td>
</tr>
<tr>
<td>MSI testing results for 5 polyps and 5 cancers</td>
<td>MSI-S</td>
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“Familial Cancer 2007: Research and Practice”
PATHOLOGY OF HYPERPLASTIC POLYPOSIS

Dr Chris Dow  
Melbourne Health Pathology  
Royal Melbourne Hospital, Parkville, Australia.

Hyperplastic polyposis (HPP) is a diagnosis rarely made by pathologists alone. The current WHO clinicopathological diagnostic criteria revolve around the number and distribution of hyperplastic polyps, treating hyperplastic polyps as a single monolithic entity. Recent advances correlating molecular changes with histopathology have changed this traditional view of hyperplastic polyps to that of a group of related “serrated polyps”, of which classical hyperplastic polyps are only a subset. In this new schema, there appears to be a progression from hyperplastic polyps, along a serrated/hypermethylation pathway, to colorectal carcinoma (CRC), via intermediate lesions including the recently described entity sessile serrated adenoma (SSA).

The serrated/hypermethylation pathway, though incompletely defined, is presumed responsible for CRC arising in HPP and SSAs are found in most HPP patients. A significant minority (10-15%) of sporadic colorectal carcinomas also appear to arise from SSAs via the serrated/methylator pathway, and many of these appear to arise from a colonic milieu of multiple late onset SSAs.

The recognition of SSAs may thus alter the future histopathological criteria for diagnosis of HPP, however, sporadic serrated/hypermethylation pathway CRC patients form a reasonably large group with a degree of confounding overlap with HPP, including multiple SSAs & hyperplastic polyps, albeit in lower numbers than HPP. The definition of true HPP patients, from the pathologist’s perspective, particularly in situations of absent or equivocal family history, may thus, paradoxically, become less clear, until the molecular lesions are more precisely defined.
Hyperplastic polyposis syndrome (HPS) is a relatively rare condition associated with multiple serrated polyps in the colon. The average age at presentation is 55 years, though ages as young as 10 have been reported. HPS affects slightly more males (56% from reported cases), has a 40% risk of CRC, and two-thirds of these CRC occur in the proximal colon. In HPS the serrated polyps demonstrate features that set them apart from the bulk of common serrated polyps often observed in the distal colon of aging populations, in that they may be unusually large, are more likely be located in at least the proximal colon, and may exhibit atypical histological architecture. HPS has been phenotypically defined by Burt and Jass as 1) at least five histologically diagnosed hyperplastic polyps proximal to the sigmoid colon, two of which are greater than 10 mm in diameter OR 2) any number of hyperplastic polyps occurring proximal to the sigmoid colon in an individual who has a first-degree relative with hyperplastic polyposis OR 3) more than 30 hyperplastic polyps of any size but distributed throughout the colon.

Approximately 20% of colorectal cancer (CRC) develops through the serrated neoplasia pathway. Serrated pathway cancers are characterized by a distinctive molecular phenotype of somatic $BRAF$ mutations and widespread concordant methylation events in CpG islands (CIMP). Both CIMP and $BRAF$ mutation are also observed in the colonic lesions from individuals with hyperplastic polyposis syndrome (HPS). HPS is rarely observed in families, however rare affected sibships suggest a recessive or co-dominant mode of inheritance. In keeping with this proposition, a family history of CRC has been reported in up to 50% of cases. Population studies have demonstrated that patients with serrated pathway cancers characterized by $BRAF$ mutation are four times more likely to have a family history of CRC than patients with common CRC. These findings are consistent with increased genetic predisposition for serrated pathway CRC in the wider population. We suggest here that HPS may result from the inheritance of two putative co-dominant alleles in approximately 1 in 2000 Anglo-Celtic individuals. Carriers of a single co-dominant allele therefore may number up to 1 in 25, and it is possible that these carriers are at increased risk of serrated pathway CRC, with implications for screening and prevention and well as for the current WHO criteria.
Tuesday 21 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

FCC Session 3: Olympic Room
Phaeochromocytoma Syndrome

Chairperson: Mike Field
TOWARDS AN UNDERSTANDING OF FAMILIAL PHAEOMOCYTOMA AND PARAGANGLIOMA

Diana E Benn\(^1\), Anne Louise Richardson\(^1\), Kathy Tucker\(^2\), Michael Croxson\(^3\), Bruce G Robinson\(^1\).

\(^1\) Cancer Genetics, Kolling Institute of Medical Research, Royal North Shore Hospital and University of Sydney; \(^2\) Hereditary Cancer Clinic, Prince of Wales Hospital and School of Medicine, University of New South Wales, Sydney; \(^3\) Department of Endocrinology, Greenlane Clinical Centre, Auckland, New Zealand.

Advances in the knowledge of the genetics of phaeochromocytoma have broadened our understanding about the mechanisms of tumorigenesis. Previously it was believed that 10% of phaeochromocytomas were associated with familial cancer syndromes, but it is now recognised that a much higher proportion of these tumours may be familial. Phaeochromocytomas are known to be component tumors of multiple endocrine neoplasia type 2 (MEN2), von Hippel Lindau disease (VHL) and rarely neurofibromatosis type 1 (NF1), and are associated with mutations in \(\text{RET}, \text{VHL}\) and \(\text{NF1}\) genes respectively. More recently it has been found that mutations in the genes encoding for succinate dehydrogenase subunit B (\(\text{SDHB}\)) and subunit D (\(\text{SDHD}\)) are causative in familial phaeochromocytoma/paraganglioma. In these cases patients may present with phaeochromocytomas and/or paragangliomas including parasympathetic associated head and neck paragangliomas. To our knowledge \(\text{SDHC}\) mutations have been associated with familial head and neck paragangliomas only. Importantly it has also been recognised that 12-24\%\(^1,2\) of patients with an apparently sporadic presentation may carry a germline mutation in \(\text{RET}, \text{VHL}, \text{SDHB}\) or \(\text{SDHD}\) genes. While the genotype/phenotype correlations are now well described for MEN2 and VHL disease, studies are only now emerging on these correlations for \(\text{SDHB}\) and \(\text{SDHD}\) mutation associated disease.

The International SDH Consortium\(^3\) showed significant differences in the estimated age of first presentation (phaeochromocytoma or head and neck paraganglioma), that is 47 years for \(\text{SDHB}\) mutation carriers and 31 years for \(\text{SDHD}\) mutation carriers (\(p=0.008\)). In addition, the value of genetic testing in these families was emphasised as subsequent screening of mutation positive relatives identified related disease in 5/38 (13\%) of asymptomatic mutation carriers. Guidelines for the screening of asymptomatic mutation carriers have been suggested but their efficacy still needs to be assessed.

During the past 6 years our laboratory has been the Australasian centre for genetic testing for genes associated with familial phaeochromocytoma and/or paraganglioma and received samples from over 500 patients or family members. A preliminary audit of these results has been performed and will be reported at the meeting.

INHERITED PARAGANGLIOMA SYNDROMES AT PRINCE OF WALES HOSPITAL RANDWICK AND OUTREACH SERVICES

Tucker K, Tyler J, Kotchetkova I, Murphy K
Hereditary Cancer Clinic, Prince of Wales Hospital.

Background: Pathogenic Mutations in SDHB and SDHD have been shown to cause both inherited Phaeochromocytomas and Paragangliomas (Pgs). Professor Bruce Robinson’s lab at Royal North Shore, under the leadership of Dr Dindy Benn, has done our mutation analysis.

Aims: To delineate the reasons for referral for SDHB and SDHD testing and to establish mutation spreading in families.

Method: All the files where SDH testing was ordered were reviewed.

Results: 19 families where SDHB or D testing was done were identified. 10/19 had a mutation identified- 8 in SDHB, 2 in SDHD and one UV in SDHD. 4 were identified externally and referred for mutation spreading/further counselling. 2/19 presented with phaeochromocytoma, 7/19 were head and neck paragangliomas(H&N)- 2 SDHD 1 SDHB 4 no mutation, 3/19 retroperitoneal paragangliomas -2 SDHB 1 no mutation, 3/19 were both H& N and retroperitoneal- 2 SDHB one no mutation, 3/19 bladder phaeochromocytomas, all SDHB 1/19 was thoracic / pericardial. SDHD UV
In 10 cases there was a family history and 9 were isolated cases. 4 mutation carriers (all SDHB) remained isolated cases despite mutation spreading and investigation of carriers. 5/9 of the no mutation found group were isolated cases. There were 7 cases of “malignancy“- 3 in bladder Pgs (all SDHB), 3 in retroperitoneal Pg (2 SDHB 1 no mutation), and one was in H&N- SDHD. 42 predictive tests were done at mean age of 38 yr with a range from 5-84yrs

Discussion: In the clinic we are only just starting to come to grips with SDH mutations. In our limited experience only one in 6 cases presenting with retroperitoneal tumour had no mutation found and that case had a family history.

Conclusion: The mutation spectrum, phenotype and penetrance of the paraganglioma syndromes is yet to be elucidated and will need collaboration and pooling of quality data.
FAMILIAL PARAGANGLIOMA: CHALLENGES IN THE GENETIC COUNSELLING SETTING

Kentwell M 1, Hossack L 1, Young M-A 1, Inder W 2, D’Costa I 1, Winship I 3,4, Harris M 5, Shanahan M 1, Benn DE 6,7, Mitchell G 1

1 Peter MacCallum Cancer Centre. 2St Vincent’s Hospital Melbourne. 3Royal Melbourne Hospital. 4University of Melbourne. 5Royal Children’s Hospital Melbourne. 6Royal North Shore Hospital. 7 University of Sydney.

The genetic basis of the Familial Paraganglioma Syndrome has only been determined recently and consequently our understanding of the clinical aspects of the syndrome, such as genotype-phenotype correlation, disease penetrance and issues of clinical risk management are still in their early stages. While Familial Paraganglioma may be a relatively rare condition, it raises important issues in the Genetic Counselling setting. We will present the Peter MacCallum Familial Cancer Centre (FCC) experience over the past 12 months, in which all (5) probands referred to our clinic with a personal history of head and neck paraganglioma have been shown to harbour a \textit{SDHB} or \textit{SDHD} mutation. We will focus on:

i. The complexity of incomplete penetrance and maternal imprinting. The literature suggests absence of syndromic features or family history does not necessarily exclude the presence of Familial Paraganglioma, as there could be an underlying germline mutation due to incomplete penetrance and maternal imprinting. Our clinical experience supports this suggestion, as 3 probands were referred as apparently sporadic cases of paraganglioma and all harboured a \textit{SDHD} mutation.

ii. Predictive genetic testing of children. A number of individuals in our families have children at 50% risk of inheriting the family SDH mutation. We will present the specific issues of informed genetic testing and clinical risk management that we have had to address in this age group.

iii. Development of multidisciplinary, tumour risk management protocols. One of the main challenges has been in establishing a surveillance program for individuals who carry a SDH mutation based on limited evidence and guidelines. This process has also highlighted the need for assistance in co-ordinating risk management for these patients who require lifelong follow up, given the complexity in risk management advice and the involvement of multiple specialists. As a result, the FCC has developed a risk management protocol with our colleagues in the head and neck and endocrinology units, and taken on a risk management co-ordination role. This role has been achieved by appointing a nurse co-ordinator to facilitate surveillance appointments, by establishing a bi-annual multidisciplinary meeting to review identified Familial Paraganglioma families with an endocrine surgeon, endocrinologist, radiation oncologist, medical oncologist, genetic counsellor, and nurse co-ordinator and providing genetic counselling support to a linked endocrinology clinic. We believe the FCC is ideally positioned in assisting patients and their specialists in co-ordinating their surveillance, and also to enhance communication between specialists, promote consistency in surveillance advice in families, maintaining up to date advice, promoting long term surveillance, and supporting families in adjusting to a genetic diagnosis. Our experience with Familial Paraganglioma and the subsequent clinical management protocol, may also be transferred to families with other rare cancer syndromes such as Li-Fraumeni, VHL, and MEN.

“Familial Cancer 2007: Research and Practice”
Tuesday 21 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

"Familial Cancer 2007: Research and Practice"

FCC Session 4: Olympic Room
Studies of Familial Renal Cancer

Chairperson: Ingrid Winship
Tuesday 21 August

FCC Session 5: Olympic Room
Notifying Relatives Without Consent

Chairperson: Gerda Evans
A germline CDH1 mutation was identified in a 39 year old woman diagnosed with signet ring cell gastric cancer. The identification of this mutation confirmed the diagnosis of hereditary diffuse gastric cancer (HDCG) in the family. Germline CDH1 mutations cause a significantly increased risk of diffuse type gastric cancer and lobular breast cancer. Germline CHD1 mutations are found in almost 50% of families with multiple cases of gastric cancer, including at least one documented case of diffuse type gastric cancer under the age of 50 years. The proband’s only significant family history was her sister diagnosed with gastric cancer at the age of 20. Both women are now deceased. The result of the genetic testing was given to the proband’s ex-husband and mother.

Genetic counselling of the proband’s ex-husband and 11 year old daughter involved discussions regarding the age at which predictive testing should be offered to children in this family, the decision whether or not to take up predictive testing by adult family members and then the management options for those found to carry the mutation, such as endoscopic screening or preventative gastrectomy. The offspring of the proband in this case were also coping with the recent loss of their mother. The probands mother attended genetic counselling and underwent predictive testing to establish the lineage of the CDH1 mutation. She was not found to carry the familial CDH1 mutation, yet informed her husband (and the reported father of the two women with gastric cancer), that she was indeed the carrier of this mutation. In addition, this patient has refused to share the information with her husband’s at-risk relatives, who are known to the Hospital.

The decision to undergo predictive testing for a CDH1 mutation is particularly difficult due to the lack of effective screening for gastric cancer and the significant long-term consequences of undergoing a prophylactic gastrectomy, which may lead to a decrease in quality of life. This decision is further complicated when considering the genetic testing and management of children. In addition, this case has raised several ethical and legal issues regarding the dissemination of information among family members and the duty to warn at-risk relatives.

* Georgina Fenton is supported by the NSW Cancer Institute
LETTING THE FAMILY KNOW - WITHOUT CONSENT

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In its exhaustive review of genetics, information, and privacy
[www.austlii.edu.au/au/other/alrc/publications/reports/96/], the Australian Law
Reform Commission recommended that the Federal Privacy Act be amended to allow
healthcare professionals to advise a patient's relatives of relevant medical information
without necessarily having the patient's consent. An amendment to the Act was passed
in 2006 giving effect to this recommendation. However, this amendment is couched in
general terms and the process of potentially breaching confidentiality without consent
raises a number of important ethical, medical, and legal issues.

The NHMRC's Human Genetics Advisory Committee was charged with the
responsibility of developing a guideline regarding the implementation of this
amendment. These guidelines would be formally viewed as an interpretation of the
amendment, and are in effect a component of the new legislation. The amendment
will not be in force until the guideline has been published.

A first draft of the HGAC guidelines has been formulated and will soon be released
for public comment. The principles will be presented and discussed in relation to
specific cases. Although the Federal Privacy Act does not apply to public sector
organisations (these being subject to State legislation), it is likely that the Act will be
the benchmark against which State legislation and medico-legal cases are considered.
Wednesday 22 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 1: Olympic Room
kConFab, AOCS and National Updates

Chairperson: Judy Kirk
THE KATHLEEN CUNINGHAM FOUNDATION CONSORTIUM FOR RESEARCH INTO FAMILIAL BREAST CANCER

G. Chenevix-Trench on behalf of kConFab
Queensland Institute of Medical Research, Brisbane

The Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab) is a multidisciplinary, collaborative framework for the investigation of familial breast cancer (http://www.kconfab.org). The primary aim of kConFab is to facilitate high-quality research by amassing a large and comprehensive resource of epidemiological and clinical data with biospecimens from individuals at high risk of breast and/or ovarian cancer, and from their close relatives.

Mutation-negative and mutation–positive multiple-case families are recruited into kConFab via the Family Cancer Clinics, if they fulfill one of the eligibility criteria (http://www.kconfab.org/epidemiology/eligibility.html). All individuals affected with breast or ovarian cancer, and their first degree relatives, are invited to participate. Collection started at the end of 1997, and I will summarize the characteristics of the 1182 families currently enrolled, from whom 10,353 blood samples and 568 fresh frozen tissue specimens have been collected, the progress and new initiatives over the last year and the plans for kConFab’s future.

The kConFab resource is available to researchers anywhere in the world, who may apply to kConFab for biospecimens and data for use in ethically-approved, peer-reviewed projects. There are more than 65 research approved projects based in Australia or overseas that depend in whole or in part on kConFab for material. There are so far 46 peer-reviewed publications that have used kConFab data or biospecimens. Results from many of these projects will be presented at the conference.
**KCONFAB FOLLOW-UP STUDY**

Kelly-Anne Phillips, John L Hopper, Michael L Friedlander, Mark A Jenkins, Sue-Anne McLachlan, Roger Milne.

**Introduction:** Prospective collection of epidemiological, and outcome data in large breast cancer family cohorts should provide less biased data than retrospective studies regarding penetrance of breast cancer and modifiers of genetic risk.

**Methods:** Since 2001 the kConFab Follow-up study has updated cancer events, risk management practices, epidemiological and lifestyle risk factors of kConFab participants every three years using self-report questionnaires. All reported new cancers and risk-reducing surgery are verified from pathology and operative reports.

**Results:** To date over 10,000 questionnaires have been mailed and the response rate is approximately 70%. Women, those affected with cancer, those married or living as married, those with a higher education level and at neither extreme of age were most likely to respond. Within the cohort, only 49% of women elect to receive their BRCA1/2 mutation result within the first 3 years of it becoming available. Of those mutation carriers who are aware of status, 11% underwent bilateral mastectomy, 29% had bilateral oophorectomy, and less than 1% took chemoprevention within the first 3 years after learning their mutation result. About 80% of carriers for whom it would be recommended were undergoing regular breast cancer surveillance, but adherence to national guidelines for ovarian cancer surveillance was much lower. Conversely, of the “true” mutation negative women within the cohort who were aware of their status, over 50% were overscreening with regard to mammography, and 10% were overscreening with regard to other modalities for breast and ovarian cancer approximately 3 years after learning their mutation negative result.

**Conclusions:** The kConFab Follow-Up Study is proceeding successfully with high participation rates. The relatively low rates of chemoprevention and risk-reducing surgery in BRCA1/2 mutation carriers in the cohort warrants further exploration as does the very high rate of overutilisation of mammography in those who are mutation negative and at average breast cancer risk. Reasons for the low uptake of disclosure of mutation results should also be investigated. Analyses of non-genetic modifiers of breast cancer risk in women at high familial risk are expected to proceed within 5 years when the data are adequately mature.

THE AUSTRALIAN OVARIAN CANCER STUDY
David Bowtell1, Georgia Chenevix-Trench2, Anna deFazio3, Dorota Gertig4, Adèle Green2, Penny Webb2 and AOCS Study Group
Peter MacCallum Cancer Centre1, Melbourne, Queensland Institute of Medical Research2, Brisbane, Westmead Institute for Cancer Research3, University of Sydney at Westmead Millennium Institute, Sydney, University of Melbourne4, Melbourne Australia.

Background
The Australian Ovarian Cancer Study (AOCS) began in 2000 as a collaborative study between researchers at the Peter MacCallum Cancer Centre (PMCC), Queensland Institute for Medical Research (QIMR), Westmead Institute for Cancer Research (WICR) and University of Melbourne. Core facilities were established for ascertainment of 1000 cases and 1000 controls from all Australian states, and to collect biospecimens, epidemiological data and detailed clinical information, with a minimum 5-year follow up.

Progress
Recruitment for AOCS is now complete, with patient recruitment ceasing on June 30, 2006. AOCS has recruited a total of 1834 women with invasive or borderline ovarian cancer, far exceeding the initial target. We have received a total of 1815 completed questionnaires and have collected 1080 fresh tumour tissue samples and 1582 blood samples. Collection of ascites is on-going, with 11 samples collected thus far. Control recruitment commenced in May 2003 is also complete. We recruited a total of 1066 control women that did not have ovarian cancer, received 1064 control questionnaires and 928 blood samples, again exceeding our initial target. As of June 30 2007, clinical follow-up had been initiated on 2408 patients nationally (this is the total number of recruited participants, including cases that were subsequently found to be benign). Post-Operative data collection has been completed on 99.9% of the total patient population. The Primary Treatment Form, including chemotherapy details and response to treatment, has been completed on 91% of cases. Data collection is ongoing and is planned to be complete in 2010. Thus far only 39 patients (2%) have been lost to follow-up, despite the fact that 30 - 40% of our patients return to regional areas for ongoing treatment.

Research Projects
In addition to case recruitment and biospecimen collection, initial funding was also awarded for three major projects within AOCS to (a) examine new epidemiologic factors associated with ovarian cancer, (b) relate germline polymorphisms in hormone metabolism and DNA repair pathways to lifetime risk of ovarian cancer, and (c) identify molecular subtypes of ovarian cancer using DNA microarray-based gene expression analysis. Analyses for these projects are well underway.

The AOCS resource is available to researchers worldwide and the number of researchers applying to access AOCS data and biospecimens is increasing. There are currently 17 projects approved or pending, including evaluation of quality of life in ovarian cancer patients (Uni of Sydney and QIMR), SNP-based CGH analysis of chemo-resistant ovarian cancer (PMCC and WICR), analysis of p53 mutation status (Hutchison/MRC labs, Cambridge, UK), and a survey of copy number change in ~200 ovarian cancer cases using SNP-based CGH analysis (PMCC in collaboration with Dana Farber).
DONOR PERSPECTIVES WITH REGARD TO DONATION OF SAMPLES AND INFORMATION: A LEGAL OVERVIEW OF KEY ISSUES

Margaret Otlowski, Professor of Law, University of Tasmania and Deputy Director, Centre for Law and Genetics

This paper provides a legal overview on key issues associated with donation of samples and information from a donor perspective. In particular, it addresses the property status of samples (if any), as well as issues in respect of consent, privacy, commercialisation and benefit sharing. The paper highlights the need for appropriate protection and safeguards for individuals, but also importantly, for understanding what donors actually think and want in terms of genetic research and the use of their samples and information. The paper seeks to emphasise the importance of transparency and accountability in the conduct of research involving samples and information in order to maximise donor participation and confidence and public trust in general in the research endeavour.

In exploring these issues, the paper seeks to draw attention to the recent revisions of the National Health and Medical Research Council (NHMRC) National Statement on Ethical Conduct in Research (2007) relevant to this area, particularly with regard to consent. Furthermore, these issues are examined against the backdrop of the important work done by the Australian Law Reform Commission and the Australian Health Ethics Committee of the NHMRC in its major inquiry into the protection of human genetic information, culminating in the Essentially Yours Report (2003) (largely endorsed by the Federal Government and in the process of being implemented).
Familial Cancer 2007: Research and Practice

Wednesday 22 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

Session 2a: Olympic Room
Molecular Pathology

Chairperson: Stephen Fox
MOLECULAR CLASSIFICATION OF BREAST CANCER. HER2 AND BASAL-LIKE PHENOTYPES, TWO AGGRESSIVE CLASSES, DIFFERENT GENES INVOLVED BUT COMMON METASTATIC PATTERNS

Frederique Penault-Llorca, Department de pathologie, centre Jean Perrin, Clermont-Ferrand, France

Breast cancer is the leading cause of mortality in women with gynaecologic cancer in developed countries, making it a significant public health issue. The incidence is in a perpetual augmentation since the last decade owing to routine mammography screening and the encouragement of prevention. In France, it affects about 1 woman out of 11 and it is responsible of 18% of cancer deaths. Recently, molecular discoveries have brought new hopes in understanding the heterogeneous pathology and in identifying new diagnostic and predictive markers. Gene-expression profiling was first used by Sorlie and Perou who classified with reliability breast tumours into 5 subtypes on the basis of its gene-expression signature: normal breast-like cancers expressing the normal components of the mammary gland; luminal A and B tumours which are estrogen-receptor (ER)-positive tumours and express genes encoding specific proteins of luminal epithelial cells; the HER-2 tumours with high level of the HER2 amplification on 17p21 and, at lastly, the basal-like subtype defined by their lack of expression of hormonal receptors (ER and PR) and HER2 expression.

Over the last few years, most attention has been given to the HER2 and the basal-like phenotype because of their specific features. These phenotypes have a relatively specific immunohistochemical (IHC) phenotype. HER2 positive tumours have been successfully targeted by trastuzumab and recently by lapatinib. Furthermore, at least 4 categories of HER2+ tumours have been described, all of them are having a direct impact for the clinical management of the patients. The basal like tumors express EGFR and share similarities with BRCA1-mutation cancer, suggesting that EGFR could be used in anti-EGFR therapies (such as cetuximab or tarceva).

The gene-expression studies have shown that those 2 phenotypes have the worst prognosis. The elevated occurrence of brain metastasis among HER2 positive patients have been the subject of extensive publications recently, aimed to understand the basis of this phenomenon (specific HER2 driven tumour aggressiveness, resistance to trastuzumab or inaccessibility of the drug to the brain metastasis, modification in the natural history of breast cancer leading to an artificially high number of brain metastasis?). But recent publications have also shown that basal-like tumours are particularly prone to metastasize to the brain especially in a BRCA1 context. This link between the 2 breast cancer molecular phenotypes suggests a possible common pathway to brain metastasis.
ABERRANT EXPRESSION OF E-CADHERIN IN LOBULAR CARCINOMAS OF THE BREAST: A MORPHOLOGICAL AND MOLECULAR ANALYSIS OF A CONSECUTIVE SERIES OF CASES

Leonard Da Silva¹, Suzanne Parry¹, Lynne Reid¹, Patricia Keith¹, Nicola Wadell², Sunil R Lakhani¹ & Peter T Simpson¹

¹ - Molecular & Cellular Pathology, Mayne Medical School, University of Queensland, Queensland Institute of Medical Research and Royal Brisbane & Women’s Hospital, Brisbane, Australia.

² - Queensland Institute of Medical Research, Brisbane, Australia.

BACKGROUND: The use of immunohistochemistry has become an essential instrument in anatomical pathology. Invasive lobular carcinoma (ILC) characteristically shows loss of expression of E-cadherin at the immunohistochemical level and so this is being increasingly used as an adjunct diagnostic tool to differentiate between lobular and ductal lesions in challenging situations. However, the lack of knowledge about the molecular biology of such a protein may lead many of us to exclude a diagnosis of lobular carcinoma in favour of one of ductal carcinoma when ‘aberrant’ positive staining is present.

METHODS: Twenty five ILC were investigated by morphology and immunohistochemistry (E-cadherin, β-catenin, ER, PgR, HER2). E-cadherin positive ILCs were subjected to comparative genomic hybridisation (CGH) and loss of heterozygosity at 16q22.1. E-cadherin gene sequencing, methylation analysis and DASL gene expression profiling were performed in different morphological components of one case.

RESULTS: Four out of 25 ILC showed positive membranous staining for E-cadherin. β-catenin was positive in two of these cases, but in both there were also neoplastic cells exhibiting aberrant (cytoplasmic/Golgi) staining. In the remaining two cases, β-catenin staining was also aberrant suggesting a non-functional E-cadherin-catenin complex. Each showed genetic changes consistent with ILC (1q+, 16q-). Case 25 exhibited solid, classic and alveolar morphologies. The solid component was positive for E-cadherin whereas the classic and alveolar areas were negative. Each component harboured an in-frame deletion in exon 7 (867del24) and loss of the wild type allele. CGH demonstrated evidence of clonal evolution from E-cadherin positive solid areas to classic and alveolar E-cadherin negative components. E-cadherin down-regulation did not occur by gene methylation. Evidence from gene expression analysis, immunohistochemistry and RT-PCR suggested E-cadherin was down-regulated through transcriptional repression via activation of TGF-β/SMAD pathways leading to differential up-regulation and phosphorylation of SMAD2 and up-regulation of transcription factors SNAIL and SLUG.

CONCLUSIONS: Positive staining for E-cadherin should not preclude a diagnosis of lobular carcinoma. Evidence from each of these cases suggests the protein is non-functional. It is necessary to be aware of the pitfall of using E-cadherin positivity to make a diagnosis of ductal phenotype where histological lobular features are present. The report out of context of E-cadherin immunostaining may lead to misclassification of tumours and, therefore, may lead to mismanagement of patients in clinical practice.
GENETIC ALTERATION IS A RARE EVENT IN BREAST AND OVARIAN CARCINOMA ASSOCIATED STROMA

Wen Qiu1,5, Anita Sridhar1, Ella Thompson1,5, Kylie Gorringe1, Ken Opeskin2, Stephen Fox3, Melanie Trivett3, Izhak Haviv4,5 and Ian G. Campbell1,5

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INTRODUCTION: Fibroblasts are a key component of human cancers. Co-culture and xenograft studies have shown that cancer associated fibroblasts (CAFs) play an important role in cancer initiation and progression. The tumour promoting ability of CAFs appears to be stable, suggesting that the cells might be programmed at the genetic or epigenetic level. Some studies have reported a high frequency of genetic aberrations in CAFs including Loss of Heterozygosity (LOH), microsatellite instability and point mutations in tumour suppressor genes and oncogenes, whereas other studies have reported very low or zero mutation rates. Consequently there is no consensus as to whether CAFs do or do not harbour cancer promoting gene mutations. To resolve this important issue, we undertook genome-wide analysis of epithelial and stromal components of 20 primary epithelial ovarian cancers, 10 primary epithelial breast cancers and five short term cultures of primary breast CAFs using the Affymetrix® GeneChip Human mapping 500K array which provides both copy number and LOH data.

METHODS: Epithelial cells and tumour-juxtaposed stromal cells less than 5mm distant from the epithelial component were manually microdissected from fresh frozen ovarian and breast carcinoma biopsies. Cultured CAFs were purified from primary breast cancers using cell-type specific markers. Purified genomic DNA was analyzed using the 500K SNP array and results were compared with the matching normal DNA from blood lymphocytes.

RESULTS: As expected a high frequency of genetic aberration was detected in the epithelial components of each tumour but LOH and copy number alterations were extremely rare in CAFs.

CONCLUSIONS: The 500K SNP array platform has been demonstrated to be a reliable and high resolution genome-analysis platform. Our data does not support the existence of a very high frequency of genetic aberrations in CAF reported in some studies. It is possible that other mechanisms, such as epigenetic changes, have a role in the tumour promoting phenotypes of CAFs.
LOSS OF HETEROZYGOSITY AT THE BRCA2 LOCUS IS A COMMON FINDING IN PROSTATE CANCER IN MEN WITH A PATHOGENIC GERMLINE BRCA2 MUTATION

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Although the prevalence of prostate cancer is higher in men with a pathogenic germline mutation in BRCA2 (and to a lesser extent BRCA1), it is unclear whether a causal relationship exists between these mutations and prostate cancer tumorigenesis. The primary aim of this study was to examine for loss of heterozygosity (LOH) in prostate cancer patients who carry a pathogenic BRCA1 or BRCA2 mutation. Secondary aims included assessment of the clinical and pathological features of prostate cancer in these individuals. Patients were selected from the kConFab cohort of 1,243 families with 137 family pedigrees identified with a known pathogenic BRCA1 or BRCA2 mutation that had at least one individual with a verified diagnosis of prostate cancer. Of the 158 male participants reported to have prostate cancer within these families, 9 were confirmed to carry a pathogenic family-specific BRCA1 mutation and 20 carried a pathogenic BRCA2 mutation by testing blood DNA. Ten cases in known carriers were excluded from the analysis due to our inability to access an archival prostate specimen. The final study cohort therefore consisted of 19 cases, five of whom were carriers of BRCA1 mutations, and 14 of BRCA2 mutations. All of the subjects belonged to families with 1-7 first degree relative and 1-5 second degree relative diagnosed with breast, ovarian or breast/ovarian cancers. The range of ages at diagnosis was 41-73 years and the mutations in the BRCA genes were varied in character, with only one being of Jewish origin. LOH analysis was performed by MLPA on DNA isolated from microdissected unstained paraffin-embedded material. LOH was confirmed by DNA sequencing. LOH at the BRCA2 locus was detected in 10/14 subjects. For the 4 subjects within the BRCA2 study cohort who were determined not to have LOH of BRCA2, microdissection of low and high Gleason score areas within the unstained section followed by MLPA is currently being performed to confirm these results. As none of the BRCA1 mutation carriers (5/5) demonstrated LOH by MLPA, methylation studies were carried out. Promoter methylation was not observed in any of the BRCA1 carriers with prostate cancer, indicating that failure to observe LOH was not due to methylation of BRCA1.

Male carriers of BRCA2 mutations in the kConFab research study were found to be at higher risk of developing prostate cancer. MLPA analysis and methylation studies suggested no role for BRCA1 in prostate cancer tumorigenesis in this cohort. Loss of the wild-type allele occurs commonly in tumors arising in BRCA2 mutation carriers, indicating a direct role for the gene in the development of prostate cancer. With the exception of very high Gleason scores no distinct clinical or histopathological features were observed.
SEBACEOUS ADENOMA AS A MARKER OF HNPCC/LYNCH SYNDROME

GK Suthers [1], J Carroll [2], A Ruszkiewicz [2], S Grist [3], J Skinner [3].
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Sebaceous adenoma is recognised as one of the extra-colonic malignancies that characterises hereditary non-polyposis colorectal cancer (HNPCC, or Lynch syndrome). We have recently received a number of requests for immunohistochemistry of the mismatch repair proteins from dermatologists who have identified sebaceous adenomas in patients. We also reviewed the immunohistochemistry of patients with sebaceous adenomas from kindreds already identified as having a heritable mismatch repair gene mutation.

In this series of 16 patients selected by various criteria, seven fulfilled the revised Bethesda criteria for testing which include sebaceous adenoma as an HNPCC-related malignancy [J Natl Cancer Inst 2004;96:261–8]. All seven patients had an abnormality of expression of one or more mismatch repair proteins in their tumours (germline mutations shown in brackets): MSH2/MSH6 in five (four MSH2 mutations identified), MSH6 alone in one (one MSH6 mutation], and MLH1/PMS2 in one (no mutation identified).

Five patients did not fulfill the revised criteria but exhibited loss of MSH2/MSH6 expression in their adenomas. Two patients were related and the older one soon developed endometrial cancer (thereby fulfilling the revised criteria); an MSH2 mutation was identified. No mutation was found in one, and two have yet to be referred for genetic counselling and testing.

Four cases did not exhibit loss of mismatch repair gene expression, did not fulfill the Bethesda criteria, and mutation analyses were not performed.

This experience suggests that sebaceous adenomas may indicate an underlying heritable mutation in MSH2 or MSH6 in patients fulfilling the revised Bethesda criteria, but the yield in other patients is likely to be low.
Wednesday 22 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 3: Olympic Room
Phenocopies in BRCA1/2 Breast Cancer Families

Chairperson: Graeme Suthers
The identification of BRCA1 and BRCA2 mutations in familial breast cancer kindreds allows genetic testing of at risk relatives. Those who test negative are usually reassured and extra breast cancer surveillance discontinued. However, we postulated that in high-risk families, such as those seen in clinical genetics centres, the risk of breast cancer might be influenced not only by the BRCA1/BRCA2 mutation but also by modifier genes. One manifestation of this would be the presence of phenocopies within BRCA1/BRCA2 kindreds. In our initial analysis we reviewed 277 families with pathogenic BRCA1/2 mutations and identified 28 breast cancer phenocopies. We assessed the relative risk of breast cancer in those testing negative using incidence rates from our local population based cancer registry. Phenocopies constituted up to 24% of tests on women with breast cancer following the identification of the mutation in the proband. The standardised incidence ratio for women who tested negative for the BRCA1/BRCA2 family mutation was 5.3 for all relatives, 5.0 for all first-degree relatives (FDRs) and 3.2 (95% confidence interval: 2.0-4.9) for FDRs in whose family all other cases of breast and ovarian cancer could be explained by the identified mutation. 13/107 (12.1%) of FDRs with breast cancer and no unexplained family history tested negative. This led us to believe that other genetic factors in these families had caused the apparent increased risk. We were particularly careful to obviate potential biases in our analyses. We would prefer that the interpretation of our article is based on the final detailed analysis of type A1 phenocopies which yielded a relative risk of three fold, equivalent to a doubling of lifetime risk. We would not suggest screening before 35 years for individuals in this category and certainly not as early as suggested in one response to our article [2]. We agree that ultimately our study needs to be confirmed in prospective analysis, before widespread change in practice. However, we are convinced that two potential biases do not contribute substantially to our original figures [2-4]. We were also concerned that identification of families for mutation testing could be because of high-risk selection finds more families with chance breast cancers [2,3]. If this were the case the ratio of first-degree relatives (FDR) with breast cancer testing negative for the family mutation before family ascertainment should be higher than afterwards. We have carried out an analysis of our enhanced dataset now containing 52 breast cancer phenocopies. The ratio of phenocopies amongst FDR remains constant at ~17% both before family ascertainment, after family ascertainment and after a mutation has been identified in the family. 11/64 (17%) cancers in FDRs were negative for the BRCA2 mutation after ascertainment, with 14/91 (15.4%) of those who developed their breast cancer before genetic testing were discharged from extra mammographic surveillance which may reduced incidence by removing the lead-time to diagnosis. The other convincing evidence now being supported by the recently identified SNPs especially FGFR2 is that the modification effect is greater for BRCA2. The phenocopy rate in BRCA2 was 19% compared to 15% for BRCA1. Estimated breast cancer incidence rates of women negative for the family mutation after family ascertainment aged 20-80 years of age was 4.65 per 1000 for BRCA2 and 3.07 for BRCA1. More detailed analysis is underway in order to help determine which family structures are likely to contain phenocopies and where the extra risk pertains [3].

In BRCA1/2 families, those individuals who test negative for the family gene mutation are usually advised that they revert to average risk. However, the presence of excess phenocopies (affected women testing negative) in one study raised the possibility that this advice may be incorrect.

The Kathleen Cuningham Consortium for Research on Familial Breast Cancer (kConFab) holds information and biospecimens from almost 1200 breast cancer kindreds from Australia and New Zealand, with an average number of 9 bloods collected per family. One of the strengths of this resource is that most of the reports of breast and ovarian cancer are verified. A causative pathogenic mutation in BRCA1 or BRCA2 has only been identified in about a third of these families. In most of these “mutation identified” families, once the causative mutation is found, further testing (“mutation spreading”) is done on the stored DNA of other affected and unaffected family members. About 6.5 tests were done on females per family, including the proband. Such spreading has been done in 186 BRCA1 families. Of 748 tested unaffected females, 230 (31%) tested positive and 518 tested negative for the family mutation. Of 517 tested women affected with breast/ovarian cancer in these BRCA1 families, 459 (89%) tested positive and 58 negative. The 58 affected who tested negative represented possible phenocopies from 39 BRCA1 families.

Spreading has also been done in 162 BRCA2 families. Of 611 tested unaffected females, 205 (34%) tested positive and 406 tested negative for the family mutation. Of 385 tested individuals affected with breast/ovarian cancer, 321 (83%) tested positive and 63 negative. The 63 affected who tested negative represented possible phenocopies from 31 BRCA2 families.

Further more detailed analysis revealed that a number of these possible phenocopies were affected individuals from the non-mutation carrying side of the family, and many of them could be excluded. Once this had been done, there were still 47 families in which there appeared to be 58 breast or ovarian cancer phenocopies on the appropriate side. However, only 22 families (13 BRCA1, 9 BRCA2) had affected cases (23 unilateral and 1 bilateral breast cancer and 2 verified ovarian cancers) testing negative that were first-degree relatives of known or obligate carriers. In these families there were a total of 113 breast cancers (55 positive, 24 negative and 34 untested) as well as 20 ovarian cancers (11 positive, 2 negative and 7 untested). Only 2 families (both BRCA2) had 2 (sibling) phenocopies. The median age at breast cancer diagnosis in phenocopies was 52 (38-81) years, compared to overall median for kConFab BRCA1 carriers 41 (22-85) years and 44 (23-76) years for BRCA2. The ovarian cancers were diagnosed at 55 and 58 years. Overall, the number of women with breast or ovarian cancer clearly not attributable to the family mutation appears to be low.

BREAST CANCER PHENOCOPIES IN BRCA-FAMILIES IN THE FAMILY CANCER CLINICS

Nicola Poplawski and the Family Cancer Clinics of Australia and New Zealand

South Australian Familial Cancer Service, Women's and Children's Hospital, North Adelaide, Australia and University Department of Paediatrics, University of Adelaide, Australia

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Smith and colleagues (Manchester, UK) recently published data suggesting that non-carrier women in BRCA-families have an increased risk of breast cancer compared to the general population.¹ They argue that this elevated risk is, in large part, due to the presence of other (non-BRCA) genetic modifiers. This conclusion has biological plausibility – breast and ovarian cancer are not 100% penetrant in women who carry a BRCA mutation and the penetrance of breast and ovarian cancer varies between families with the same BRCA mutation.

Subsequent correspondence has raised the possibility that the findings of Smith et al are the consequence of ascertainment bias.²⁻⁴ It is suggested that it is premature to recommend additional screening or chemoprevention for unaffected women who do not carry their family BRCA mutation (beyond the population recommendations for women in their age group).

We present a preliminary small data set from a number of Australasian Family Cancer Clinics, describing the Clinics’ experience of breast cancer in non-mutation carriers from BRCA-families. This will provide a valuable discussion point for our session on breast cancer phenocopies in BRCA-families.

THE EPIDEMIOLOGIST’S POINT OF VIEW

David Goldgar

Smith et al. recently reported a significantly elevated risk of breast cancer among non-carriers in breast cancer families in which a BRCA1 or BRCA2 mutation had been identified through clinical testing. The authors found an elevated risk of approximately 5-fold, which, if true, has considerable impact on the counseling and clinical management of women testing negative for the mutations found in their family. However, it is possible that much of this effect is due to ascertainment bias, as families with more cases (both carriers and non-carriers) are more likely to seek (and be eligible for) genetic testing. In order to examine this possibility, we simulated genetic and phenotype data under a model with a constant rate of such phenocopies and analyzed the effect of different modes of ascertainment on the observed relative risk of disease in non-carrier individuals in carrier families. Our results indicated that the effects of family selection could explain much of this observed risk. The bias is particularly evident when the penetrance of the disease allele is low and when stringent criteria for testing are employed. For example, for a gene with population disease risks of 1% in non-carriers and 10% in carriers, if only families with 2 or more affected individuals are tested/analyzed, the apparent risk to non-carriers in positive families is 3.4%, compared to 1.1% when all carrier families are included. To address the issue of if modifying effects truly confer an increased risk of cancer in BRCA1/2 negative individuals in BRCA positive families will likely require large prospective studies of such individuals.
Wednesday 22 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 4: Olympic Room
Breast Cancer Profiling

Chairperson: Ian Campbell
MOLECULAR STRATEGIES IMPROVE THERAPEUTIC DECISIONS
Laura J. van 't Veer
The Netherlands Cancer Institute, Amsterdam, The Netherlands

Breast cancer starts as local disease and may metastasise to the lymph nodes and distant organs. Currently, at primary diagnosis, prognostic markers are used to assess the likelihood that the transition to systemic disease has occurred. The prevailing model of metastasis reflects this view, suggesting that metastatic capacity is a late acquired event in tumourigenesis. Others have proposed that breast cancer is intrinsically a systemic disease. New molecular technologies, such as DNA microarrays, support the notion that metastatic capacity might be a breast tumour's inherent feature. These data have important implications for prognosis prediction, our understanding of metastasis, and may provide the new prognostic markers that are urgently needed to identify patients with breast cancer who are at the highest risk for developing metastases.

Improving our understanding of the molecular mechanisms of the etiology and metastatic process might also improve clinical management of the disease. According to the widely held model of metastasis, rare subpopulations of cells within the primary tumour acquire advantageous genetic alterations over time, which enable these cells to metastasise and form new solid tumours at distant sites. Many studies have challenged this 'genetic-selection' model of metastasis in the past, but only recent data obtained by gene expression profiling of human breast carcinomas have received broader attention. DNA microarray studies reported that primary breast tumours that developed metastases could be distinguished by their gene expression profile from those, that remained localised. The data imply that the metastatic capacity of 'poor prognosis' breast tumours might be acquired by mutations at much earlier stages of tumourigenesis than previously assumed. The genetic make-up of a patient, i.e., SNP composition is also likely to determine the biological nature of the developed tumour. Some preliminary findings indeed substantiate this hypothesis.

Recent results show that the molecular program established in a primary breast carcinoma is highly preserved in its distant metastasis. These findings further strengthen the idea that metastatic capability in breast cancer is an inherent feature, and is not based on clonal selections.

Management of breast cancer tailored to individual patients by molecular strategies is of benefit to the patients.

Figure 1 | An integrative model of breast cancer metastasis. Oncogenic mutations occurring in a breast stem cell (red) can cause the transformation to a breast cancer stem cell, generating 'poor-prognosis' tumours (orange). Mutations occurring in differentiated progenitor cells (yellow) might form a non-metastatic 'good-prognosis' breast carcinoma (pink). In the metastatic poor-prognosis tumours, under the influence of stromal fibroblasts, only the population of breast cancer stem cells has the ability to metastasize. There might be variant cancer stem cells that differ in their tissue selectivity for metastasis, expressing an additional tissue-specific profile (for example: green, bone; purple, lung). At the site of metastasis, the disseminated cancer stem cells would again induce a similar stromal response as in the primary breast tumour.

EXPRESSION PROFILING REVEALS POTENTIAL SUBGROUPS WITHIN FAMILIAL BREAST TUMOURS

Waddell\(^1\) N., kConFab Investigators\(^2\), Simpson\(^1,3\) P., DaSilva\(^1,3\) L., Holland\(^1\) H., Grimmond\(^4\) S. Lakhani\(^1,3\) S., and Chenevix-Trench\(^1\) G.

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2. Peter MacCallum Cancer Centre, Melbourne, Australia
3. Department of Pathology and School of Molecular and Microbial Sciences, University of Queensland Pathology, Brisbane, Australia
4. Institute for Molecular Biosciences, Brisbane, Australia

Approximately 5% of women with breast cancer have a strong family history of disease but only 30-45% of severely affected families carry mutations in the predisposing genes \(BRCA1\) and \(BRCA2\), therefore other genes (‘\(BRCAx\)’) must be responsible for the burden of disease. Expression profiling has shown that \(BRCA1\), \(BRCA2\) and \(BRCAx\) tumours have distinct expression profiles, and that there might be two classes of \(BRCAx\) tumours (Hedenfalk 2001 and 2003). If replicated, this finding would have important implications because stratification of \(BRCAx\) tumours into subgroups would focus the search for breast cancer associated genes by reducing heterogeneity.

We have used Illumina bead arrays to expression profile a cohort of 56 fresh frozen breast tumours from kConFab (all of which have >50% neoplastic component), including 15 \(BRCA1\), 13 \(BRCA2\), 18 \(BRCAx\) and 10 who have not had full mutation screening of \(BRCA1/2\). Unsupervised hierarchical clustering of all samples separates tumours into estrogen positive and negative branches. As expected, \(BRCA1\) tumours, which have a basal-like phenotype, cluster with the ER and PR negative tumours. Further analysis identified potential subgroups of \(BRCAx\) tumours which may aid in the identification of novel \(BRCA\) genes. However, to our surprise we found more convincing evidence for subtypes of \(BRCA2\) tumours. Data analysis using ANOVA identified 86 transcripts that discriminate the \(BRCA2\) subtypes (\(p = 0.0005\)) and principle components analysis and hierarchical clustering of these genes can clearly separate the \(BRCA2\) tumours. Furthermore the \(BRCA2\) tumours can be separated into their subgroups using the 70 gene prognosis signature of van ’t Veer et al\(^1\), suggesting that the subgroups may be clinically relevant. We have selected candidate classifiers and now plan to use antibodies to analyse further \(BRCA2\) tumours by IHC on tissue microarrays, and are designing a DASL panel to confirm both \(BRCA2\) and \(BRCAx\) subgroups in a larger cohort of formalin-fixed paraffin-embedded tumours.

CLINICAL CLASSIFICATION OF BRCA1 AND BRCA2 MISSENSE SUBSTITUTIONS – POTENTIAL LOW-MODERATE RISK VARIANTS IDENTIFIED BY MULTIFACTORIAL ANALYSIS, FUNCTIONAL STUDIES, AND GERMLINE MICROARRAY PROFILING.

Amanda B. Spurdle1, Nic Waddell1, Sue Healey1, Sunil R. Lakhan1,2, Paul K. Lovelock1,3, Myth Mok4 Fergus J. Couch5, Daniel J. Farrugia5, Anette Ten Haaf1, Anna Marsh1, Julie Johnson1, Logan Walker1, Gongora M6, kConFab Investigators2, Beric R. Henderson4, Sean Grimmond6, Sean V. Tavtigian8, Georgia Chenevix-Trench1, David E. Goldgar9, Melissa A. Brown9

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2 Molecular & Cellular Pathology, School of Medicine, University of Queensland, Brisbane, Australia.
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6 Institute for Molecular Biosciences, University of Queensland, Brisbane, Australia.
7 Peter MacCallum Cancer Centre, Melbourne, Australia.
8 International Agency for Research on Cancer, Lyon, France.
9 Department of Medical Informatics, University of Utah, Salt Lake City, USA.

Many of the DNA sequence variants identified in the breast cancer susceptibility gene BRCA1 remain unclassified in terms of their potential pathogenicity. Both multifactorial likelihood analysis and functional approaches have been proposed as a means to elucidate likely clinical significance of such variants, but analysis of the comparative value of these methods for classifying all sequence variants has been limited. Multifactorial likelihood analysis, which incorporates sequence conservation, co-inheritance, segregation, and tumor immunohistochemical analysis, may improve classification of variants. Nevertheless, some variants remain classified, and at least some of these may represent moderate-risk variants that do not exhibit the characteristics of classical high-risk BRCA1/2 mutations. Revised multifactorial analysis of a series of unselected BRCA1/2 unclassified variants identified in kConFab families showed the BRCA1 variants R1699Q and A1708V to have posterior probabilities of causality of 54% and 69%, only moderately suggestive of increased risk. In support of this, results from functional analyses suggest that both these variants have only partial functional activity, in terms of number of assays showing defects in BRCA1 function and the level of activity measured by different assays - foci formation in response to DNA damage, transcriptional transactivation activity, and centrosome amplification. These data indicate that a range of functional studies are required to identify variants with partially compromised function. Further evidence for low-moderate risk variants comes from parallel studies assessing the value of lymphoblastoid cell line (LCL) gene expression data to predict the significance of clinically unclassified variants in the breast cancer predisposition genes BRCA1 or BRCA2. We compared post-ionising radiation expression profiles of LCLs from breast cancer-affected women carrying pathogenic truncating or missense mutations in BRCA1 or BRCA2, to familial breast cancer cases with no BRCA1/2 pathogenic mutations (BRCAx). A subset of BRCAx individuals who carried rare BRCA1/2 sequence variants considered to be neutral or of low clinical significance (as determined previously by multifactorial analysis), differed in expression from the remaining BRCAx LCLs and clustered with high risk pathogenic BRCA1/2 mutations. This suggests that some variants which do not exhibit overt characteristics of pathogenic mutations (inheritance, tumour histopathology, evolutionary conservation) may nevertheless have similar expression profiles at the germline level. Alternative statistical approaches will be required to assess the cancer risk associated with variants that do not exhibit the characteristics of classical pathogenic mutations.
IDENTIFICATION OF NOVEL MUTATION TARGETS IN THE BREAST CANCER SUSCEPTIBILITY GENE \textit{BRCA1} AND GENES THAT ENCODE REGULATORS OF \textit{BRCA1}

Juliet French\textsuperscript{1}, Jodi Saunus\textsuperscript{1}, Sue Mei Wong\textsuperscript{2}, Brooke Brewster\textsuperscript{1}, Stacey Wardrop\textsuperscript{1}, Sean Tavtigian\textsuperscript{3}, Melissa Southey\textsuperscript{3}, Peter Leedman\textsuperscript{5}, Nicholas Proudfoot\textsuperscript{2} and Melissa Brown\textsuperscript{1}.

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\textsuperscript{2}Sir William Dunn School of Pathology, University of Oxford, England.
\textsuperscript{3}International Agency for Research on Cancer, Lyon, France.
\textsuperscript{4}Department of Pathology, University of Melbourne, Victoria, Australia.
\textsuperscript{5}Laboratory of Cancer Medicine, Western Australian Institute of Medical Research, Perth, Australia.

It is becoming increasingly clear that regulatory mutations as well as coding region mutations play an important role in disrupting the function of tumor suppressor genes and that germline regulatory mutations in such genes are likely to play an important role in conferring susceptibility to cancer. The \textit{BRCA1} gene maps to human chromosome 17q21 and is associated with risk of breast and ovarian cancer. Our group is interested in identifying the sequence elements and factors that control the regulation of \textit{BRCA1} expression and thus may be targets of disease-associated mutations. In particular we are interested in identifying novel transcriptional control elements in \textit{BRCA1} and examining the role of these sequences, and proteins that bind to these sequences, in gene looping mediated transcriptional initiation. We are also interested in the role of elements in the 3’UTR that regulate mRNA stability and translation and are targets of micro RNAs. In this presentation we provide evidence that sequences in the extragenic and intonic regions of \textit{BRCA1} gene possess regulatory activity and are involved in gene looping. We also show that elements in the 3’UTR and are involved in regulating \textit{BRCA1} mRNA dynamics, in normal processes such as cell cycle progression. We show that several proteins bind to the \textit{BRCA1} 3’UTR, including the RNA binding protein HuR and that HuR can regulate the expression of \textit{BRCA1}. We will also present a preliminary analysis of the \textit{BRCA1} 3’UTR for miRNA target sites.
Thursday 23 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 5:
National Updates

Chairperson: Graham Mann
Thursday 23 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 6a: Jackson Landy Room
Attitudes Towards Cancer Prevention Strategies

Chairperson: Bettina Mesier
PREVENTIVE STRATEGIES FOR HBOC: CONTEXTUAL INTERPRETATION OF INTERNATIONAL DISCREPANCIES IN ATTITUDES AND BEHAVIOURS

Claire Julian-Reynier

The availability of genetic tests for BRCA gene mutations prompted cancer geneticists to give information about genetic risk and to assess many women with a personal or family history of breast or ovarian cancer to inform them of preventive measures. Previous results have shown in comparative settings international variations in women’s theoretical acceptability of the preventive strategies available. A literature review will confront theoretical attitudes and actual behaviours of women at risk for HBOC observed in different countries. Several hypotheses to explain these results will be assumed among which the cultural component will be discussed more in depth. Contextual factors intervening in the different health care systems organisations, in doctors' and in patients' behaviours will be highlighted.
BARRIERS TO THE DISCUSSION OF BREAST CANCER CHEMOPREVENTION IN AUSTRALIAN FAMILY CANCER CLINICS

Louise Keogh¹, Doreen Rosenthal¹, John Hopper², Kelly-Anne Phillips³,⁴

¹Key Centre for Women’s Health in Society, School of Population Health, University of Melbourne, ²Centre for MEGA Epidemiology, School of Population Health, University of Melbourne. ³Division of Haematology and Medical Oncology, Peter MacCallum Cancer Centre, ⁴Dept of Medicine, University of Melbourne; Victoria, Australia

Chemoprevention is an effective strategy to prevent breast cancer, reducing risk by up to 50%. Over a thousand women at high risk of breast cancer present to familial cancer clinics across Australia each year, motivated by a desire to ameliorate their cancer risk. However, our previous work suggests that only about 1% of Australian high-risk women are prescribed tamoxifen or raloxifene for breast cancer prevention. The U.S. literature suggests that there is reluctance among family physicians to prescribe tamoxifen due to concerns about the side effects and lack of experience with the drug. The aims of this study were to determine what risk management advice is offered to women at high risk of breast cancer when they attend a Family Cancer Clinic in Australia, to identify the framework that clinicians and counsellors use to advise women on how to reduce their breast cancer risk, and to identify perceived barriers to advising high risk women about the option of chemoprevention. Focus groups were conducted with staff at five Family Cancer Clinics in three Australian capital cities to explore the provision of risk management advice. Focus groups were audiotaped and transcribed with participants assigned pseudonyms to protect their privacy. Transcripts were then analysed to identify themes. Preliminary results will be presented, identifying the frameworks and approaches adopted by clinicians and counsellors, and the main barriers to the discussion of chemoprevention in the family cancer setting.
ATTITUDES TO USE OF SURGICALLY REMOVED TISSUE FOR RESEARCH

Lesley Andrews 1 Blendine Shlaimon 2 Bernie Tuch 3
1 Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick, New South Wales
2 Department of Obstetrics and Gynaecology, University of Sydney, New South Wales
3 Diabetes Transplant Unit, Prince of Wales Hospital, Randwick, New South Wales

Background
Further development in stem cell research is required for it to progress to clinically useful therapeutic cloning. Sources of oocytes for this research, and ultimately clinical use, are being sought. One option is to extract oocytes from prophylactically removed ovarian tissue from women who are at high genetic risk of ovarian cancer. In order to determine if this is an acceptable option to such women, a survey of attitudes to this type of research was conducted.

Methods
Patients of the Prince of Wales Hospital Hereditary Cancer Clinic at high risk of ovarian cancer, who have been offered prophylactic oophorectomy, were mailed a questionnaire regarding their attitudes to stem cell research, therapeutic cloning, and donation of tissue for such research.

Results
215 eligible women were mailed the survey. 107 returned responses. The remainder were either lost to contact or declined to participate. Of the 107 respondents, 99% felt that stem cell research should continue with the current or lesser restrictions, and that therapeutic cloning would be or may be an important source of future health care. No participant thought this type of research should be discontinued. 75% would definitely agree to having surgically removed tissue used for research and the other 25% would agree if they knew exactly what the research involved. None said they definitely would not agree to surgically removed tissue being used for research. The same results were found when asked specifically about surgically removed ovaries.
Similar findings applied to tissue in general and ovaries in particular being specifically used for stem cell research (73% and 26%), with one respondent saying they definitely would not agree to such use.
A slightly lower number said they would definitely agree to body tissue or ovaries being used for therapeutic cloning (70%), or would do so if they knew exactly what the research involved (29%). Again, one respondent said they would definitely not agree to use of their tissue for therapeutic cloning.
Qualitative responses indicated that this group of women were strongly in favour of stem cell research with a view to therapeutic cloning. Concerns included commercialisation, adherence to regulations and assurances that the ovaries would not be used for human cloning.

Conclusions
These findings indicate that women undergoing prophylactic oophorectomy may be a willing source of oocytes for stem cell research and therapeutic cloning. Subsequently, a study to utilise prophylactically removed ovaries to develop techniques to harvest and mature oocytes for stem cell research has been commenced.
Thursday 23 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 6b: Olympic Room
DNA Repair and Cancer

Chairperson: Melissa Brown

“Familial Cancer 2007: Research and Practice”
DNA DAMAGE INDUCED BY CHRONIC INFLAMMATION IS A MAJOR CONTRIBUTOR TO CARCINOGENESIS

Leona D. Samson, Biological Engineering Department, Biology Department and Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Chronic inflammation increases cancer risk. While it is clear that cell signaling elicited by inflammatory cytokines promotes tumor development, the impact of DNA damage production by inflammation associated reactive oxygen and nitrogen species (RONS) has not been directly tested. RONS induce DNA damage that can be recognized by Alkyladenine DNA Glycosylase (Aag) to initiate DNA base excision repair. Using a mouse model of episodic inflammatory bowel disease we show that Aag-mediated DNA repair prevents colonic epithelial damage, and strongly suppresses colon tumorigenesis. Importantly, RONS-induced DNA base lesions recognized by Aag, accumulated to higher levels in Aag-deficient animals following stimulation of colonic inflammation. Finally, as a test of the generality of this effect we show that Aag-deficient animals are more susceptible to developing precursors of gastric cancer after infection with *Helicobacter pylori*. These data demonstrate that the repair of DNA lesions formed by RONS during chronic inflammation is important for suppressing carcinogenesis.
In 2006 we described 4 families with heterozygous PMS2 mutations. We now report an update on these 4 families, present 3 additional PMS2 families (see table). **Family A** [1021delA]: A family meeting Amsterdam II criteria for HNPCC. The proband experienced adenocarcinoma of the colon (AdCRC) at 49 and oesophageal cancer at 51. **Family B** [equivocal variant 137G>T (S46I)]: The proband, who meets Bethesda 2.1, experienced metachronous AdCRC at 32 and 68. Family history (FmHx) is limited to two second degree relatives with malignant melanoma at 65 and breast cancer at 78. The limited available evidence suggests the variant is likely to be pathogenic. **Family C** [del exon 10]: The proband, who meets Bethesda 2.1, experienced synchronous AdCRC and squamous cell carcinoma of the rectum at 60. FmHx is limited to a third degree relative with breast cancer at 40. **Family D** [1414A>T (L472X)]: A proband meeting Bethesda 4.2 who experienced EAdEndo at 43. Her FmHx of cancer includes 1 breast cancer at 64, and 2 unknown cancers at 40 and 60. **Family E** [736_741del16ins11]: A family meeting Amsterdam II criteria for HNPCC. The proband experienced AdCRC at 37. **Family F** [1031_1032insA (I611X)]: A proband meeting Bethesda 4.1, who experienced AdCRC at 39. Neither the paternal FmHx of AdCRC, CR polyps and transitional cell carcinoma of the bladder, nor the maternal FmHx of AdCRC, breast and gastric cancers meet Amsterdam or Bethesda criteria. Parental mutation status is unknown. **Family G** [736_741del16ins11]: A proband who experienced a AdCRC (caecal) at 57. The only known FmHx is of 2 second degree relatives (1 maternal, 1 paternal) who experienced lung cancer.

**Conclusion:** In our experience, endometrial and colorectal carcinomas associated PMS2 mutations show selective loss of the PMS2 protein. PMS2 mutation families may have extensive CRC histories (e.g. high risk CRC families A, E, F), or concerning individual CRC histories with minimal FmHx (families B, C, D), but unremarkable family histories are also seen (family G).

<table>
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<th>IHC: abn</th>
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DNA MISMATCH REPAIR DEFICIENCY IN EARLY-ONSET ENDOMETRIAL CARCINOMAS

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*Joint senior authors

Lynch Syndrome (hereditary non-polyposis colon cancer) is an autosomal dominant condition which predisposes to early-onset cancer predominantly of the colorectum and endometrium. A major advance in the diagnosis of Lynch Syndrome has been the establishment of immunohistochemistry techniques to detect deficiency of four DNA mismatch repair (MMR) proteins, MLH1, MSH2, MSH6 and PMS2 in tumour samples. The risk of developing endometrial cancer within Lynch Syndrome is equal to or in excess of that associated with colorectal cancer in female mutation carriers, identifying young onset EC as a “sentinel cancer” for this disorder. The aim of this study was to determine the prevalence of MMR deficiency in endometrial cancers presenting at or below age 50. Eighty-three cases of early-onset (<51 years) endometrial carcinoma (EC) were identified from the Queensland Gynaecological Oncology Registry. Histology review and tumour classification into endometrioid or other sub-types was performed by a single pathologist (MCC). Tumor sections were stained for the MMR proteins MLH1, PMS2, MSH2 and MSH6. MLH1 negative tumours were assessed for MLH1 promoter methylation using a MethyLight assay. Loss of one or more MMR proteins was seen in 23/83 tumors (27.7%): 12 showed loss of MLH1 and PMS2, nine tumors MSH2 and MSH6, and two tumors MSH6 alone. MLH1 methylation was observed in 8/12 (66%) tumors with absent MLH1 & PMS2. The MMR+ve group comprised 55 endometrioid, two mixed tumors, two MMMTs, and one SCC whilst the MMR-ve group consisted of 20 endometrioid, two mixed tumours, and one MMMT. More MMR-ve tumors had intermediate or high nuclear grade than MMR+ve (71% vs. 43%; p<0.05), moderate or high overall grade (65% vs. 42%; p <0.05) and the MMR-ve endometrioid ECs were more frequently FIGO grade 2 or 3 (35% vs. 9.9%; p<0.025). No association was seen between MMR status and histotype, tumor location, focality, lymphovascular invasion, serosal involvement, extrauterine tumor, node status or FIGO stage and mitosis. There was a trend towards more TIL-positive ECs in the MMR-ve group (52.4% vs. 32.8%, p = 0.07) but no association between MMR and the presence of PTLs. This study suggests that Lynch Syndrome is associated with approximately one in five cases of early onset endometrial cancer, and that routine immunohistochemistry testing for MMR proteins, particularly in cases with moderate to high nuclear and FIGO grades would result in significant clinical benefit to patients and their relatives with Lynch Syndrome.
DO WE NEED BOTH MSI AND IHC FOR TRIAGING HNPCC?

Z Rudzki¹, S Grist², K van Diemen³, G Suthers³.

¹ Institute of Medical and Veterinary Science, Adelaide SA; ² Flinders Medical Centre, Bedford Park SA; ³ Familial Cancer Unit, Women's & Children's Hospital, North Adelaide SA.

Mutations in four DNA mismatch repair genes, MSH2, MSH6, MLH1 and PMS2, cause HNPCC (Lynch syndrome). Bi-allelic mutations in any one of these genes in a cancer cause microsatellite instability (MSI). However, MSI is not exclusive to HNPCC, and can occur in non-familial cancers. Loss of expression of these genes can be assessed by immunohistochemistry (IHC) and indicate which gene is mutated. A screening system using MSI and IHC reduces the number of genes requiring analysis to identify the mutation responsible for HNPCC. We asked whether it is necessary to use both MSI and IHC to guide mutation analyses, or whether IHC alone can suffice.

We commenced MSI testing in 1998, and IHC was introduced progressively over the next six years as antibodies became available. Of the 1,031 MSI tests performed, we have IHC results for all 4 mismatch repair proteins in 240 patients. Of these, 99 exhibited MSI and 141 did not. Of the 99 samples with MSI, only 61 demonstrated abnormal IHC. All of the samples with abnormal IHC exhibited MSI. Hence MSI is not a good predictor of IHC, but abnormal IHC is a good predictor of MSI.

The “gold standard” against which these tests should be compared is the identification of heritable mutations. We have found a total of 74 mutations in patients with tumours exhibiting MSI: 32 in MLH1, 28 in MSH2, 9 in MSH6, and 5 in PMS2. In each case, there were one or more instances of cancers exhibiting MSI and abnormal IHC documented in the family. We have documented two cases in which carriers had tumours which did not have abnormal IHC. One had an adenoma (MSH2 nonsense mutation); the other had CRC (MLH1 missense mutation). In each case, there were related carriers whose cancers had both abnormal IHC and MSI.

We conclude that abnormal IHC is an excellent indicator of MSI, and also identifies the gene most likely to be mutated. The addition of MSI usually provides no additional information to assist in identifying the mutation responsible for the clinical diagnosis. We no longer include MSI in routine screening for HNPCC.
Thursday 23 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 7: Olympic Room
Ovarian Cancer

Chairperson: Anna de Fazio
PATHOLOGY OF FAMILIAL OVARIAN CARCINOMA

C. Blake Gilks MD FRCPC, Dept of Pathology, University of British Columbia and Vancouver General Hospital, on behalf of the Cheryl Brown Ovarian Cancer Outcomes Unit, Vancouver Canada, and the UK Familial Ovarian Cancer Registry.

Background: Taking into account recent progress in histopathological classification of ovarian surface epithelial carcinomas, we sought to address the following questions:
1. Do ovarian carcinomas arising in patients with BRCA1 or BRCA2 mutations have sufficiently distinctive histopathological features that routine histopathological examination can be used to stratify patients for genetic counseling and mutation testing?
2. Do tumors with epigenetic silencing of BRCA1 share similar pathological features with tumors having BRCA1 or BRCA2 mutations?

Materials and Methods: BRCA1 and BRCA2 mutations, and epigenetic silencing of BRCA1 through promoter hypermethylation were characterized in an consecutive series of 49 cases of ovarian carcinoma from our hospital. We assessed CD8 +ve tumour infiltrating lymphocytes (TILs) in these tumours. We also reviewed 66 cases from the United Kingdom Familial Ovarian Cancer Registry where germ-line BRCA1 and BRCA2 mutation status was known.

Results: The histopathology of the 49 cases was as follows: 38 high grade serous/undifferentiated, 5 endometrioid, 4 clear cell, and 2 low grade serous carcinomas. Nine tumours had BRCA1 mutations (8 germline and 1 somatic), and three had BRCA2 mutations (2 germline and 1 somatic). Thus 10 of 49 (20%) cases were associated with a germline BRCA1 or BRCA2 mutations. Epigenetic silencing of BRCA1 was found in an additional 9 of 49 (18%) tumors. All tumours showing BRCA1 or BRCA2 mutation, or BRCA1 epigenetic loss were high grade serous/undifferentiated carcinomas. The presence of CD8 positive TILs was significantly more common among the cases with BRCA1 mutation or BRCA1 epigenetic silencing, compared to tumours lacking BRCA abnormalities (18/19 versus 11/19, p = 0.008). In the independent series of cases from the UK Familial Ovarian Cancer Registry, there were 49 tumours in the high grade serous/undifferentiated group and 31 of these were from patients with BRCA1 or BRCA2 mutations. Of 17 tumours other than high grade serous/undifferentiated, there were three from mutation carriers. This difference between high grade serous/undifferentiated carcinomas (31/49, 63 %), and carcinomas of other types (3/17, 18 %), is significant (p=0.002).

Conclusions: BRCA1 or BRCA2 mutation is sufficiently common in ovarian carcinomas of high grade serous/undifferentiated type that consideration should be given to referring all such patients for genetic counseling. BRCA1 loss through epigenetic silencing also occurs in high grade serous/undifferentiated carcinomas, so that most tumors of this type will have loss of BRCA1 or BRCA2 through genetic or epigenetic means.
Molecular Subclassification of Ovarian Cancer by Expression Profiling

Richard Tothill1, Anna Tinker5, Joshy George1, Bob Brown6, Stephen Fox1, Bianca Locandro1, Kathryn Alsop3, Dariush Etemadmoghadam1, Nadia Traficante1, Sian Fereday1, AOCS Study Group1,2,3,4, Anna De Fazio7 and David Bowtell1

1 Peter McCallum Cancer Centre, Melbourne, Australia 2 Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Sydney, Australia 3 Queensland Institute for Medical Research, Brisbane, Australia 4 University of Melbourne, Melbourne, Australia 5 Vancouver Cancer Centre, British Columbia Cancer Agency, Canada 6 Melbourne Pathology, Melbourne Australia 7 Westmead Institute of Medical Research, Sydney, Australia

Ovarian cancer is a histologically and clinically diverse disease. One of our key research interests is to apply genomics technologies, such as microarray expression profiling, to examine the molecular heterogeneity of ovarian cancer. We have profiled over 300 ovarian carcinoma specimens on expression microarrays (Affymetrix U133 Plus2) from the Australian Ovarian Cancer Study (AOCS) cohort providing an unparalleled perspective on the transcriptional landscape of ovarian cancer. Focusing on the two most common histological subtypes of serous and endometrioid tumours we have used unsupervised analysis methods to identify six discrete molecular subtypes of ovarian carcinoma. Two molecular subtypes are defined by either serous LMP or low grade endometrioid tumours, harbouring expression signatures reflecting low cell proliferation, cell type and key mutations in known oncogenes. Four predominantly high grade subtypes appear to be driven, at least in part, by two large gene clusters associated with stromal or immune cell elements. Two subtypes displayed differential expression for genes synonymous with an activated or reactive fibrotic stroma. The gene cluster is enriched with genes associated with extracellular matrix, matrix remodelling, markers of activated myofibroblasts (ACTA2, FAP), cell adhesion, cell signalling and angiogenesis. Over-expression of genes associated with immune cells, immune signalling and response is evident within two subtypes, one with and one without the stromal signature. Markedly reduced expression of immune responsive genes was found within another subtype, which is defined by a mesenchymal signature and lacks expression of known tumour antigens or markers such as MUC1 and MUC16 (CA125). We used laser capture microdissection, immunohistochemistry and morphological review to further characterise our findings from the microarray data. We found significant associations with levels of desmoplasia and immune cell infiltration across the subtypes. Using clinical follow-up from the AOCS we have found that there are significant differences in time to relapse and overall survival between the subtypes. The most striking survival differences existed between those tumours with high stromal response (poor survival) and those with high immune cell response but lack the high stromal signature (good survival). These findings highlight the importance of the tumour microenvironment in ovarian carcinoma and the need to develop new therapeutic agents that either inhibit or promote host cell interactions and function.
BIOMARKER AND FUNCTIONAL ROLES OF THE SERINE PROTEASE, KLK7 AND ITS NOVEL SPLICE VARIANT IN EPITHELIAL OVARIAN CANCER

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Ovarian cancer is the leading cause of death worldwide from gynaecological cancers. The five-year survival rate is just 25-35%, due to presentation at a late stage. Therefore, a better understanding of the tumour biology and factors involved in the development and progression of ovarian cancer is needed. Kallikrein-related peptidases (KLKs/KLKS) have been found to be aberrantly expressed in ovarian cancer and are emerging as valuable biomarkers. Multiple mRNA splicing has been reported in the majority of KLK proteases, some of which show differential expression between normal and cancerous tissues.

The aims of this study were to examine expression of KLK7-253 and its N-terminally truncated isoform KLK7-181 in normal and malignant ovaries and determine the role of KLK7-253 and KLK7-181 proteins in ovarian cancer using stably over-expressing cells and in vitro functional assays. The two KLK7 mRNA transcripts differ at their 5’UTR, exon 2 and 3’UTR sequences. The longer form encodes a 253 amino acid pre-pro enzyme of KLK7 (KLK7-253), whereas the shorter 5’-truncated transcript encodes a protein of 181 amino acids which is missing the pre-pro sequence and the histidine of the catalytic triad and is therefore catalytically inactive.

Using quantitative RT-PCR analysis we found that levels of both KLK7 transcripts were higher in serous ovarian carcinomas and serous ovarian cancer cell lines compared with normal ovarian tissue and ovarian surface epithelial cell lines. Following stable transfection with KLK7-253V5/His and KLK7-181V5/His SKOV3 ovarian cancer cells show altered cell morphology being more rounded than the spindle shaped vector-only clones and parental cells. KLK7-253 was cytoplasmically localised and secreted into the media as expected, whereas KLK7-181 was localised throughout the entire cell suggesting a different function. Functionally SKOV3:KLK7-181 over-expressing cells showed a significant increase in proliferation compared with vector only controls and the SKOV3:KLK7-253 clones. Clones were more resistant to the chemotherapy drug cisplatin. They also showed less adherence to vitronectin and fibronectin when compared with SKOV3:KLK7-181 clones suggesting that they secrete a factor that degrades these extra cellular matrix compounds.

These results suggest that both of these kallikrein 7 isoforms have potential as ovarian tumour markers and could play a role in ovarian cancer development and progression. This is the first study to show that a KLK isoform (KLK7-181) may have a non-protease biological function. In addition, the increased cisplatin resistance observed for KLK7-253 expressing cells suggests a potential role in chemosensitivity which is very important for ovarian cancer patient prognosis.
Collagen and Calcium Binding EGF Domains 1 (CCBE1), A Novel Tumour Suppressor Gene in Ovarian Cancer?

Caroline Barton\(^1\), Brian Gloss\(^1\), Wenjia Qu\(^1\), Neville Hacker\(^2\), Robert Sutherland\(^1\), Susan Clark\(^1\), Philippa O’Brien\(^1\)

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Epithelial ovarian cancer is the 5\(^{th}\) most common cause of death from cancer in women, with a five year survival rate of only 40%. As the molecular basis of ovarian cancer remains ill-defined, the aim of this study was to further understand the genetic changes underlying ovarian tumour development. To identify candidate tumour suppressor genes (TSGs) in epithelial ovarian cancer, we used transcript profiling and bioinformatic analysis to determine genes with highly downregulated expression in serous ovarian cancers, the most common histological type of ovarian carcinoma, compared to normal ovaries. One such gene, \(CCBE1\), maps to 18q21.32, a region identified by allelic imbalance studies in ovarian cancer as harbouring a TSG(s). \(CCBE1\) encodes a protein of unknown function that is highly expressed in normal ovary. It contains an EGF-like calcium-binding domain and a collagen repeat and may therefore play a role in the interaction between the ovarian surface epithelium and the extracellular matrix. Reduced/loss of expression compared to normal ovarian surface epithelium was confirmed in ovarian cancer cell lines and serous carcinoma tissue extracts by qPCR. Treatment of ovarian cancer cell lines with the methyltransferase inhibitor 5-aza-2’deoxyctydine and the histone deacetylase inhibitor trichostatin A resulted in re-expression of \(CCBE1\) mRNA and suggests that epigenetic modification of the promoter region is involved in \(CCBE1\) gene silencing. Furthermore, using methylation-specific PCR and real-time dissociation methylation analysis, we showed methylation of the \(CCBE1\) promoter in 6/8 (75\%) ovarian cancer cell lines and 8/50 (16\%) serous ovarian cancers. Knockdown of \(CCBE1\) using siRNA enhances proliferation of ovarian cancer cells and re-expression of \(CCBE1\) inhibits the colony forming ability of epithelial cancer cells. In addition, preliminary data suggests re-expression of \(CCBE1\) decreases the ability of epithelial cancer cells to migrate and invade. Taken together, our findings show that \(CCBE1\) is susceptible to methylation-induced gene silencing and may suppress cancer cell growth, migration and invasion, and is thus a candidate TSG with a role in the development and progression of ovarian cancer.
CHARACTERISATION OF microRNA GENES IN OVARIAN CANCER

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MicroRNAs are a class of small non-coding RNAs that negatively regulate protein expression by targeting messenger RNA. In recent years, several studies have provided evidence that implicate microRNA in the development and progression of cancer. Aberrant expression has been observed across several tumour types, and specific microRNA profiles, based on histological subtype, hormone receptor status, vascular invasion and proliferation index, suggests further understanding of these expression patterns will be of diagnostic and prognostic value.

To this end, an integrated genomics approach is being used to identify those microRNA that may play a role in the pathogenesis of ovarian cancer. In addition to the generation of genome-wide microRNA expression profiles, candidate microRNA genes are being investigated for genetic and epigenetic aberrations that might alter their expression. High Resolution Melt analysis and direct sequencing have been used to detect somatic mutations in cancer-related microRNA genes, in a panel of serous, endometrioid and mucinous ovarian tumours. Analysis is ongoing, however findings to date suggest somatic mutation in these genes is rare.
Thursday 23 August

Session 8a: Olympic Room
Genetic Epidemiology

Chairperson: John Hopper
A GENOME-WIDE ASSOCIATION STUDY OF BREAST CANCER


Genome-wide association studies (GWAS) have recently become possible due to the development of high-density SNP arrays capable of genotyping at >500,000 loci. We conducted a GWAS of breast cancer by genotyping 528,173 SNPs in 1,145 postmenopausal women of European ancestry with invasive breast cancer and 1,142 controls from the Nurses’ Health Study. We identified four SNPs in intron 2 of FGFR2 (which encodes a receptor tyrosine kinase and is amplified or overexpressed in some breast cancers) that were highly associated with breast cancer and confirmed this association in 1,776 affected individuals and 2,072 controls from three additional studies. Across the four studies, the association with all four SNPs was highly statistically significant (P(trend) for the most strongly associated SNP (rs1219648) = 1.1 x 10(-10); population attributable risk = 16%). Four SNPs at other loci most strongly associated with breast cancer in the initial GWAS were not associated in the replication studies. Our summary results from the GWAS are available online in a form that should speed the identification of additional risk loci. Large-scale replication studies in a multi-stage design are ongoing to validate additional breast-cancer associated loci. The FGFR2 locus and other loci have been identified as associated with breast cancer by the Breast Cancer Association Consortium (BCAC). These novel loci associated with breast cancer have implications for risk stratification, and potentially for understanding of carcinogenetic mechanisms and possible interventions.
DO BREAST CANCER PREDISPOSITION GENES ALSO PREDISPOSE TO PROSTATE CANCER?

Fabrice Odefrey¹, Melissa Southey¹, Andrea Tesoriero¹, Dallas English²,³, Gianluca Severi³, John Hopper², Graham Giles³

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Population-based studies of the genetic epidemiology of breast and prostate cancer have shown that only a small proportion of the familial aspects of these diseases can be explained by what is currently known about their causes. A very small proportion of early-onset breast cancer is due to mutations in genes such as BRCA1 and BRCA2 that convey an extremely high increased risk (10 to 20-fold) of breast cancer. An even smaller proportion (~5%) of early-onset prostate cancer is due to mutations in BRCA2 that convey a similarly high increased risk of prostate cancer.

In recent years, common polymorphisms have been tested for their potential role in cancer predisposition with predominantly null findings. Most recently, results from the Breast Cancer Association Consortium (BCAC) using an extremely large genome-wide association study have found that common SNPs in the genes FGFR2, TNRC, MAP3K1, LSP1, H19 and on 8q convey a very small increased risk of breast cancer and explain <4% of the population polygenic variance (Easton et al., 2007).

We have investigated the possibility that, similar to BRCA2, these common genetic variants that convey breast cancer risk also convey risk of prostate cancer. We have tested common genetic variations within BRCA2 (ex10 c.1342 C>A and ex27 c.10204 A>T), and the newly identified “BCAC” SNPs that have been implicated in breast cancer susceptibility, in our Risk Factors for Prostate Cancer case-control study (831 cases and 736 controls). We genotyped these SNPs using high resolution melt curve analysis and Taqman assays.

We found no association between BRCA2 c.1342 C>A or the c.10204 A>T variant and prostate cancer risk. We sequenced the carriers of the 10204 A>T for the linked, BRCA2 6503 delTT protein truncating mutation and found none. The analysis of the “BCAC” SNPs is near completion and the data will be presented.

**MDR-1 SINGLE NUCLEOTIDE POLYMORPHISMS AND OVARIAN CANCER RELAPSE AND SURVIVAL**

Sharon E. Johnatty¹, Jonathan Beesley¹, Sian Fereday², AOCS Management Group, Paul Harnett³, Anna deFazio³ and Georgia Chenevix-Trench¹

Queensland Institute of Medical Research,¹ Brisbane, Australia, Peter MacCallum Cancer Center,² Melbourne, Australia, Westmead Institute for Cancer Research,³ University of Sydney at the Westmead Millennium Institute, Westmead Hospital Sydney, Australia.

The human multidrug resistance (MDR)-1 gene belongs to the superfamily of ATP binding cassette transporters, and encodes a 170 kDa transmembrane transporter, P-glycoprotein (PGP) which recognizes and extrudes a broad range of toxic substrates including most anti-cancer drugs. Several single nucleotide polymorphisms (SNPs) have been identified in the coding region of the MDR-1 gene, the most common being the synonymous C1236T and C3435T, and the nonsynonymous G2677T/A. While the functional and pharmacological significance of these SNPs remain controversial, the 2677T/A and 3435T alleles have been shown to be associated with reduced PGP expression. A recent study of the G2677T/A SNP and response to chemotherapy in 53 ovarian cancer patients showed an allele dose-dependent correlation between the mutant T/A alleles and a good response to paclitaxel treatment (Green et al, Clin Cancer Res, 12: 854-9, 2006). We present here the results of association studies between the MDR-1 G2677T/A SNP and overall survival in 915 women with invasive epithelial ovarian cancer as well as progression-free survival in a subset of 309 cases who received a minimum of 4 cycles of paclitaxel (135 or 175 mg/m²) and carboplatin (AUC 5 or 6). Date of disease progression was determined using CA125/RECIST criteria and time to event was calculated from the date of histological diagnosis to the date of relapse or death. We estimated crude survival probabilities using the Kaplan-Meier product limit method, and univariate and multivariate hazard ratios (HRs) and 95% Confidence Intervals (CIs) adjusting for surgical stage (FIGO criteria), histological subtype and residual disease following laparotomy (none vs. <1 cm vs. >1 cm) using Cox proportional hazards models. The crude Kaplan-Meier progression-free survival among combined homozygote and heterozygote 2677T/A carriers was significantly better compared to homozygote G2677 carriers ($p = 0.001$). Likewise, a significant trend in better progression-free survival was associated with increasing T or A mutant alleles ($p_{\text{trend}} = 0.004$). However, in adjusted analysis, heterozygote and homozygote 2677T/A carriers showed a non-significant progression-free survival advantage compared to homozygote wildtype carriers (adjusted HR$_{T/A \text{ carriers}} = 0.74$, 95% CI = 0.54 - 1.03, $p = 0.07$). We found no association between overall survival and MDR-1 genotypes in either univariate or multivariate hazard models ($p \geq 0.1$). Our findings support the hypothesis that the mutant allele of the G2677T/A SNP is inversely associated with progression-free survival, but may require a larger sample to definitively assess its impact on response to treatment and overall survival.
TALCUM POWDER, CHRONIC PELVIC INFLAMMATION AND NSAIDS IN RELATION TO RISK OF EPITHELIAL OVARIAN CANCER

Melissa A. Merritt\textsuperscript{1,2}, Adèle C. Green\textsuperscript{1}, Christina M. Nagle\textsuperscript{1}, Penelope M. Webb\textsuperscript{1*}, Australian Cancer Study (Ovarian Cancer) and Australian Ovarian Cancer Study Group

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Chronic inflammation has been proposed as the possible causal mechanism that explains the observed association between certain risk factors such as the use of talcum powder (talc) in the pelvic region, and epithelial ovarian cancer. To address this issue we evaluated the potential role of chronic local ovarian inflammation in the development of the major subtypes of epithelial ovarian cancer. Factors potentially linked to ovarian inflammation were examined in an Australia-wide case-control study comprising 1576 women with invasive and low malignant potential ovarian tumours and 1509 population-based controls. We confirmed a statistically significant increase in ovarian cancer risk associated with use of talc in the pelvic region (adjusted odds ratio [OR] 1.17, 95% confidence interval [CI]: 1.01-1.36) that was strongest for the serous and endometrioid subtypes although the latter was not statistically significant (adjusted OR=1.21, 95%CI 1.03-1.44 and OR=1.18, 95%CI 0.81-1.70, respectively). Other factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection and mumps) were not associated with risk but, like others, we found an increased risk of endometrioid and clear cell ovarian cancer only among women with a history of endometriosis (adjusted OR=1.85, 95%CI 1.02-3.38 and OR=2.66, 95%CI 1.31-5.44, respectively). Regular use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) was inversely associated with risk of low malignant potential (LMP) mucinous ovarian tumours only. We conclude that on balance chronic inflammation does not play a major role in the development of epithelial ovarian cancer.
Thursday 23 August

Session 8b: Landy Jackson Room
Psychosocial Impact of Genetic Testing

Chairperson: Melanie Price
COGNITIVE AND BEHAVIOURAL ADJUSTMENTS TWO YEARS AFTER AN INCONCLUSIVE GENETIC TEST RESULT IN A COHORT OF HBOC AFFECTED WOMEN

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Little is known about how women who receive an inconclusive result from BRCA1/2 testing interpret their result. No long term prospective study is available on cognitive and behavioral adjustment they develop to cope with this uncertainty.

Our objective was to explore affected women’s cognitive and behavioral adjustments to an inconclusive BRCA1/2 test result and to what extent these adjustments were linked.

This study was carried out on 83 women with personal and familial breast/ovarian history of cancer, who received inconclusive result to genetic testing two years before. Self-administered questionnaires were prospectively collected. Here we present a qualitative analysis of open written commentaries obtained on risk perception and diffusion of information and when relevant, of corresponding closed questions.

61.4\% women made commentaries on genetic predisposition. There did not differ for socio-demographic characteristics from those who did not. We observed three types of reactions to inconclusive result: 11 women coped with the uncertainty of the result, 9 women turned out the meaning of the “negative result” into a certainty and considered they were not at a higher risk anymore, last group (6 women), continued to be convinced to be at-risk, given the personal history of cancer. In every group, behaviours they declared to adopt towards diffusion of information to family and preventive strategies will be presented.

Our findings show the precautions that practitioner must take to ensure that women with inconclusive results understand that their family remains, most of the time, at a high risk of developing breast/ovarian cancer.
MEN ARE FROM MARS AND WOMEN ARE FROM VENUS? MEN FROM HIGH RISK BREAST CANCER FAMILIES SAY IT’S NOT ROCKET SCIENCE.

Lobb, E.A.,1,2 Hallowell, N., 3 Kristjanson, L., 1 Meiser, B.,4,5 Butow, PN.2

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5Prince of Wales Hospital Clinical School, University of New South Wales, Australia.  
2The Medical Psychology Research Unit, School of Psychology, University of Sydney, NSW, Australia.

Background: Although the incidence of breast cancer in men is small, the under-representation of men in familial cancer clinics, the under-representation of women at risk because of a paternal family history of breast cancer, and the potential for the daughters of male mutation carriers to develop breast cancer, makes understanding the attitudes and experiences of Australian men from high-risk breast cancer families a research priority.

Purpose: This Australian study of men from high risk breast cancer families aimed to: a) examine expectations of genetic counselling; b) assess breast cancer genetics knowledge; (c) assess risk perception; (d) identify interest in genetic testing; (e) investigate cancer specific anxiety and (f) assess information and decision-making preferences. Sample: Men who had a family history consistent with a dominantly inherited susceptibility to breast cancer were invited to participate. Men who were at low risk, were partners of women from high-risk breast cancer families and who had a previous diagnosis of cancer were excluded.

Methods: A self-administered questionnaire was mailed to 479 men through kConFab in December 2005. 211 questionnaires were returned (48% response rate). Results: Forty five percent (45%) of the men had previously attended a family cancer clinic and 72% of these attended at the suggestion of a family member. The majority (79%) reported telling their partner they were going to the clinic and 44% of men went to the clinic alone.

Expectations: Multivariate analyses showed that men who had 2 or more relatives who had died from breast or ovarian cancer were 6.6 times more likely to want information than those who had no relatives who had died (p = 0.017). Men with higher levels of cancer related anxiety were significantly more likely to want information (p = ≤0.001). Knowledge: Men who were professionally employed (p=0.016) and had attended a familial cancer clinic (p=≤0.001) had significantly higher levels of knowledge of cancer genetics. Genetic Testing: The majority of men (88%) said they discussed their decision to have genetic testing with a family member. The majority would “definitely not” want assistance in telling their family about their test result (54%) and 39% said “probably not”. Less than half the men wanted a collaborative decision making process (47%). Conclusions: This presentation will provide an overview of data from men from high risk breast cancer families. There are striking similarities with the data from our previous study with women from high risk families. Implications for clinical practice will be discussed and caution is urged that male stereo-types do not reinforce a bias about gender responses.
FAMILIAL CANCER CENTRES EMERGING LIFETIME ROLE FOR INDIVIDUALS WITH MUTATIONS IN CANCER PREDISPOSITION GENES.

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The Familial Cancer Centre’s (FCC’s) main role has traditionally focussed on the identification of families at high hereditary risk for developing cancer. The role of an FCC beyond the point of identification of high risk families and individuals has, in the past, remained rather limited. However, in response to emerging evidence regarding the long term considerations of cancer risk management and psychosocial issues affecting people at high hereditary cancer risk, FCC’s are beginning to widen their ongoing role and services to these families.

The FCC at the Peter Mac Callum Cancer Centre is committed to providing long term clinical care and support to individuals with hereditary cancer syndromes. The Peter Mac Callum Cancer Centre has already established Multidisciplinary Cancer Risk Management Clinics for individuals with hereditary breast/ovarian or colorectal cancer syndromes. However not all individuals with mutations in cancer predisposition genes attend these clinics. Therefore, the FCC at Peter Mac is establishing a formalised follow up programme to provide ongoing cancer risk management advice and psychosocial support to all mutation carriers. The structure of the programme will be informed by research into services provided to mutation carriers at a number of international Familial Cancer services. This research has been supported by the Victorian Quality Council’s Victorian Travelling Fellowship Program. The first phase of visiting with the target institutions will be completed by July 2007.

This presentation will:

- Outline the carrier follow up programmes for mutation carriers offered by a selected number of international Familial Cancer services.
- Describe the evidence that suggests the clinical need for implementation of these follow up programmes
- Propose a framework for the development and implementation of programs in a local setting
- Highlight areas that require further research and evaluation when developing a follow up programme for mutation carriers.
A RANDOMISED CONTROLLED TRIAL OF A DECISION AID FOR INDIVIDUALS CONSIDERING GENETIC TESTING FOR HEREDITARY NON-POLYPOSIS COLORECTAL CANCER (HNPCC)

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5) Hunter Family Cancer Service, Hunter New England Health, NSW, Australia;
6) Familial Cancer Service, Westmead Hospital, Westmead, NSW, Australia.

Purpose: To measure the effectiveness of a tailored decision aid designed to help individuals make informed decisions about genetic testing for hereditary non-polyposis colorectal cancer (HNPCC) risk. Methods: 150 individuals were randomized to receive the decision aid or a control pamphlet at the end of their first genetic counselling consultation. As of May 2007, 102 of the 150 individuals randomised (68%) completed the first questionnaire 1 week post-consultation, while 74 (49.3%) completed the 6-month follow-up questionnaire. Results: While the decision aid had no significant effect on post-decisional regret or actual genetic testing decision, the trial showed that participants who received the decision aid had significantly lower levels of decisional conflict (ie. uncertainty) about their genetic testing decision ($\chi^2(1)=9.36; P=0.002$) and were more likely to be classified as having made an informed choice about genetic testing ($\chi^2(1)=4.49; P=0.034$), than participants who received the control pamphlet. The decision aid appeared to help male participants in particular, such that men who received the decision aid had significantly higher knowledge levels about genetic testing compared to men who received the control pamphlet, while no such differences were found for women ($\chi^2(2)=6.09; P=0.048$). Clinical implications: A decision aid for individuals considering genetic testing for HNPCC is an effective intervention to reduce uncertainty and assist individuals to make an informed choice about genetic testing for HNPCC after genetic counseling. Research implications: Previous research in the more common hereditary cancer syndromes such as hereditary breast/ovarian cancer can be successfully translated into other, rarer hereditary cancer syndromes, potentially saving resources and expanding our current knowledge base into new areas.

*We wish to thank the following additional members of the Australian Genetic testing Decision Aid Collaborative Group: Centre for Genetics Education, Sydney (K.Barlow-Stewart); Familial Cancer Service, Westmead Hospital, Sydney (G.Fenton, A.Goodwin, P.Zodgekar); Hereditary Cancer Clinic, Prince of Wales Hospital, Sydney (L.Andrews, E.Christian, J.Koeler, A.Overkov, M.Peate, J.Tyler, B.Warner), Hunter Genetics, Newcastle (T.Dudding, M.Gleeson, C.Groombridge, S.O’Donnell, A.Spigelman); Macquarie University (C.McMahon, A.Taylor); Peter McCallum Cancer Institute, Melbourne (L.Hossack, M.Kentwell, M.Young); Royal Melbourne Hospital, Melbourne (C.Aragona, R.D’Souza, C.Gaff, L.Hodgkin); University of Sydney (P.Butow).
Friday 24 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 9: Olympic Room
Modifiers and Moderate Risk Genes

Chairperson: Georgia Chenevix-Trench
MODELLING GENETIC SUSCEPTIBILITY TO BREAST CANCER: RISK PREDICTION MODELS, VALIDATIONS AND GENETIC MODIFIERS OF RISK.

Antonis C Antoniou, Paul Pharoah, Georgia Chenevix-Trench and Douglas F Easton

Several breast cancer susceptibility genes have been identified to date. \textit{BRCA1} and \textit{BRCA2} account for the largest proportion of the so far explained excess familial risk of breast cancer. We have previously derived the BOADICEA model of genetic susceptibility to breast cancer in which susceptibility is explained by mutations in \textit{BRCA1} and \textit{BRCA2}, together with a polygenic component reflecting the joint effects of genes with small effects. We have now updated BOADICEA, using additional family data from two UK population based studies of breast cancer and data on families with \textit{BRCA1} and \textit{BRCA2} mutations. We have also extended the model to account for the risk of second cancers and the risks of other \textit{BRCA1} and \textit{BRCA2} associated cancer types, including prostate, pancreatic and male breast cancer. BOADICEA, BRCAPRO and the Manchester scoring system, are currently been evaluated in a series of 2000 families from cancer genetics clinics in the UK. We will present the results on several properties of the models including calibration, discrimination and accuracy in predicting \textit{BRCA1} and \textit{BRCA2} mutation carrier probabilities.

The risk of breast cancer in \textit{BRCA1} and \textit{BRCA2} carriers has been found to be higher in women with a strong family history of the disease, suggesting that other genetic factors modify these risks. BOADICEA allows for this effect by incorporating a large number of risk alleles which modify the risk of breast cancer in \textit{BRCA1} and \textit{BRCA2} mutation carriers. However, the results from studies of genetic modifiers have so far been conflicting and reported genetic modifiers have not been reliably replicated. Recently, several common variants identified through genome-wide association studies have been shown to increase the risk of breast cancer in the general population. Such variants also provide plausible candidates as risk modifiers in \textit{BRCA1} and \textit{BRCA2} mutation carriers. To evaluate whether the recently identified SNPs in \textit{FGFR2}, \textit{TNRC9} and \textit{MAP3K1} also modify the breast cancer risk in \textit{BRCA1/2} mutation carriers we genotyped approximately 11000 mutation carriers in the Consortium of Investigators of \textit{Modifiers of BRCA1 and BRCA2} (CIMBA) for these SNPs. The data are currently being analysed and the results as well as the implications for risk predictions will be discussed.
VARIANTS OF ATM, BRIP1, PALB2 AND CHEK2 MAY HAVE HIGH PENETRANCE FOR MOST WOMEN WITH A STRONG FAMILY HISTORY

GB Byrnes\textsuperscript{a}, MC Southey\textsuperscript{b}, JL Hopper\textsuperscript{a}

\textsuperscript{a} Centre for Molecular, Environmental, Analytic and Genetic Epidemiology, The University of Melbourne, Victoria, Australia
\textsuperscript{b} Department of Pathology, The University of Melbourne, Carlton, Victoria, Australia

In the quest to discover genetic variants that influence disease risk, it is important to remember what risk estimates mean for the individual carrier. Association studies, in which cases and controls are screened for genetic variants, usually present findings in terms of multiplicative relative risks; i.e. the risk for carriers divided by that for persons similar in every way except carriage of the variant. There are complications if cases are selected due to having a family history because these familial cases may be enriched for carriers due to the variant’s effect on risk for both the individual and their relatives. Pre-test risk depends on family history, is largely independent of the individual variant being measured, and may differ greatly between individuals. For an individual, the consequences of screening will depend on context and a variant should not be pre-judged as being universally “low risk” based on its relative risk for the average person.

We detail this argument for women who present for counselling at breast cancer family clinics who typically bear a high risk prior to any genetic testing due to their family history. In particular, we consider the implications from studies of ATM, BRIP1, PALB2 and CHEK2, and indicate how detection of mutations in these genes may be of considerable clinical consequence in terms of absolute risk of breast cancer (i.e. penetrance).

This has immediate implications for Australian breast cancer family clinics and research using kConFab.
Friday 24 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Session 10: Olympic Room
Risk Prediction Models

Chairperson: Gillian Mitchell
RISK PREDICTION - LOGISTIC REGRESSION MODEL

David Goldgar, Medical Informatics and Family and Preventive Medicine, The University of Utah, Genetic Epidemiology, Salt Lake City, USA

Over the last decade, a number of methods for predicting risk of carrying a BRCA1 or BRCA2 mutation and consequently the risk of developing breast or ovarian cancer by a given age have been developed. One of the most frequently used is the simple scoring system developed by Gareth Evans and colleagues at St. Mary’s Hospital in Manchester UK (1,2). This so-called Manchester Score (MS) method has been shown to perform quite well in a number of studies of series of clinically ascertained families when compared to other methods (3,4). In addition, as part of a study of unclassified variants based on a large dataset of ~70000 tested individuals from Myriad Genetics Laboratories, Inc. we developed a new logistic regression (LR) model encompassing 24 parameters based on personal and family history of cancer of the tested proband (5). This model has yet to be applied in an independent dataset. In order to test both of these models in independent datasets, we applied them to a set of approximately 600 breast cancer families that comprise the kConFab resource on familial breast cancer in Australia. Receiver-Operator Curves (ROC) were constructed for each method and the areas under the curve (AUC) calculated using the result of the genetic testing of the most tested individual in each family. Crude preliminary analyses showed that the LR model performed slightly better for BRCA1 compared to the MS with areas under the curve of 0.80 vs. 0.74. In contrast, for BRCA2 both models were less predictive than for BRCA1 and did not differ in their ability with AUCs of ~0.66 for both models. Each of these methods will be further analyzed using an updated and standardized dataset and compared to several other risk prediction algorithms.
Boadicea and kConFab
Antonis Antoniou, Cambridge University, UK
Tyler Cuzick and kConFab
Graham Mann, Westmead Institute for Cancer Research, Sydney
BRCAPRO and kConFab

James Dowty and the kConFab genotype-prediction team, The University of Melbourne

BRCAPRO is a computer program which estimates a woman’s chance of carrying a mutation in BRCA1 or BRCA2 from her family history of breast and ovarian cancer. It is used in genetic counselling and in epidemiological research to prioritise women for mutation testing. This talk will describe the new features of the 2006 version of BRCAPRO and will discuss the strengths and weaknesses of the program. These points will be illustrated through its performance on the kConFab families.
GOODBYE “ADELAIDE”, HELLO “MANCHESTER”.

J Armstrong, N Poplawski, K van Diemen, G Suthers.
Familial Cancer Unit, Women’s & Children’s Hospital, North Adelaide SA 5006
e-mail: graeme.suthers@cywhs.sa.gov.au

In the last decade, the SA Familial Cancer Service has tested 827 patients for mutations in the BRCA1 and BRCA2 genes. We developed an arbitrary set of clinical “Adelaide criteria” to select patients for testing, and 729 patients fulfilled one or more of these criteria (see companion presentation). But with changes in the types of families being referred, the positive predictive value (“detection rate”) of these criteria has fallen from >30% in the late 1990s to 10% in 2006. We have re-assessed our families using the Manchester scoring system [J Med Genet 2004;41:474–480] to assess the performance of this triage tool in our patient population.

In the cohort of 827 cohort of patients, the combined Manchester score (i.e. sum of BRCA1/2 scores) performed well, with the area under the ROC curve being 0.80. When compared to the Adelaide criteria, it can provide comparable sensitivity with much higher specificity i.e. fewer tests of patients without identifiable mutations. The Table summarises the predicted outcome of testing using various threshold Manchester scores.

<table>
<thead>
<tr>
<th>Threshold Manchester score</th>
<th>Either Score &gt;=10</th>
<th>Combined Score &gt;=10</th>
<th>Combined Score &gt;=15</th>
<th>Combined Score &gt;=20</th>
<th>Adelaide criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test volume (% of 827)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>729 (88%)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>352 (43%)</td>
<td>751 (91%)</td>
<td>498 (60%)</td>
<td>329 (40%)</td>
<td>494 (94%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>83%</td>
<td>97%</td>
<td>88%</td>
<td>82%</td>
<td>94%</td>
</tr>
<tr>
<td>Pos. predict value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13%</td>
</tr>
<tr>
<td>Neg. predict value</td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
<td>18%</td>
</tr>
<tr>
<td>Mutations found</td>
<td>116 (83%)</td>
<td>136 (97%)</td>
<td>123 (88%)</td>
<td>115 (82%)</td>
<td>131 (94%)</td>
</tr>
<tr>
<td>Presymps done</td>
<td>416 (89%)</td>
<td>456 (98%)</td>
<td>430 (92%)</td>
<td>416 (89%)</td>
<td>441 (95%)</td>
</tr>
<tr>
<td>Mutations missed</td>
<td>24 (17%)</td>
<td>4 (3%)</td>
<td>17 (12%)</td>
<td>25 (18%)</td>
<td>9 (6%)</td>
</tr>
<tr>
<td>Presymps missed</td>
<td>49 (11%)</td>
<td>9 (2%)</td>
<td>35 (8%)</td>
<td>49 (11%)</td>
<td>24 (5%)</td>
</tr>
</tbody>
</table>

The average combined Manchester score per family has fallen from >30 in the late 1990s to 18.0 in 2006, and the positive predictive value has declined from 40% to 18% in this period (using 15 as the threshold).

On balance, the Manchester score is a better triage tool for families now being referred to our service, and it is time for the “Adelaide criteria” to be retired. But the Manchester score is not perfect, and it will be necessary to remain vigilant and consider other tools to select patients as our knowledge and patient population change.
Mutation testing for *BRCA1* and *BRCA2* needs to be targeted to families with a high likelihood of harbouring a pathogenic mutation. To date, a few models are available to assist in the prioritisation of samples, and some involve time-consuming computerisation. We devised a simple system of scoring families using data from mutation screening of a cohort of samples. DNA samples from affected family members from 422 Non-Jewish families with a history of breast and/or ovarian cancer were screened for the presence of *BRCA1* mutations, with 318 being screened for *BRCA2* by whole gene screening techniques. A second dataset of 182 samples from another region was also used. Using a combination of results from screening and the family history present in mutation negative and positive kindreds, a simple scoring system was devised for the prediction of pathogenic mutations. This was the further validated on a set of 257 samples and compared against existing models. The scoring system includes a cutoff point at 10 points for each gene. This equates to >10% probability of a pathogenic mutation in *BRCA1* and *BRCA2* individually. The manual scoring system was as or more sensitive than all the other models, including BRCAPRO, but had a far higher specificity and a better positive predictive value at both a 10% and 20% prediction for the presence of mutations.

Since this time the Manchester system has been compared to existing models in a number of countries, but not always appropriately (sometimes Jewish samples were included). The system has continued to perform well on sensitivity and specificity at the 10 and 20% thresholds even against the new computer model BOADICEA. We have updated analysis on over 1700 non-Jewish samples screened in Manchester.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Ovarian</th>
<th>Male breast</th>
<th>All families</th>
</tr>
</thead>
<tbody>
<tr>
<td>40+</td>
<td>58/76 (76%)</td>
<td>7/9 (78%)</td>
<td>72/99 (72%)</td>
</tr>
<tr>
<td>35-39</td>
<td>32/59 (54%)</td>
<td>4/5 (80%)</td>
<td>32/59 (54%)</td>
</tr>
<tr>
<td>30-34</td>
<td>23/50 (46%)</td>
<td>4/9 (45%)</td>
<td>45/99 (45%)</td>
</tr>
<tr>
<td>25-29</td>
<td>26/96 (27%)</td>
<td>3/9 (33%)</td>
<td>55/188 (29%)</td>
</tr>
<tr>
<td>20-24</td>
<td>24/94 (25%)</td>
<td>2/9 (22%)</td>
<td>63/280 (22.5%)</td>
</tr>
<tr>
<td>15-19</td>
<td>6/60 (10%)</td>
<td>1/15 (6.6%)</td>
<td>43/361 (12%)</td>
</tr>
<tr>
<td>12-14</td>
<td>1/30 (3.3%)</td>
<td>0/6 (0%)</td>
<td>16/330 (5%)</td>
</tr>
<tr>
<td>&lt;12</td>
<td>0/0</td>
<td>0/3</td>
<td>4/279 (1%)</td>
</tr>
<tr>
<td>Total</td>
<td>160/445 (36%)</td>
<td>21/65 (32%)</td>
<td>330/1715 (19%)</td>
</tr>
</tbody>
</table>
Wednesday 22 August

A combined meeting of kConFab, Australian Ovarian Cancer Study (AOCS), & the Family Cancer Clinics of Australia and New Zealand

“Familial Cancer 2007: Research and Practice”

Poster Session
7.30 – 10.30pm
The Pavilion at Blue Gum Point
USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINES (CAM) IN INDIVIDUALS FROM MULTIPLE-CASE BREAST CANCER FAMILIES IN THE KATHLEEN CUNINGHAM CONSORTIUM FOR RESEARCH INTO FAMILIAL BREAST CANCER (kCONFAB)

Kelly-Anne Phillips1, Mark Jenkins2, Joanne McKinley1, Kathryn Field1, Michael Friedlander3, Prue Weideman1, John Hopper2, Geoff Lindeman4, Sue Anne McLachlan1,5, Louise Keogh2, kConFab Investigators1

1Peter MacCallum Cancer Centre, Vic; 2University of Melbourne, Vic; 3Prince of Wales Hospital, NSW; 4Royal Melbourne Hospital, Vic; 5St Vincent’s Hospital, Vic.

Background: CAM is very frequently utilized by cancer patients, but there are few data regarding use of CAM in well individuals with high cancer risk.

Methods: A mailed, self-report questionnaire, which included questions on CAM use, was administered to unaffected men and women approximately 6 years after their recruitment into kConFab. Possible predictors of CAM use were assessed. Proportions were compared and Fisher’s exact test was used to determine if associations between possible predictors and CAM use were significant.

Results: Questionnaires were mailed to 2435 subjects, 1693 (70%) responded (65% of males 73% of females). 296 were excluded from this analysis because they were not at high risk for cancer (spouses or negative for family mutation and knew their result), leaving 1397 subjects with mean age 46.5 years. 676 of 1397 (48%) subjects reported CAM use, 11% of users indicated their use was specifically to prevent cancer. Of the 676 subjects who reported CAM use, 78% used vitamins and herbs, (10% of these specifically for cancer), 45% used physical therapies (2% for cancer), 42% used mind/body therapies (4% for cancer), and 41% used special diet (9% for cancer). A wide range of different CAM were used, but that most frequently used to prevent cancer was green tea (4% of all CAM use) and that most frequently used overall were vitamins/supplements (52% of all CAM use). Predictors of CAM use were: being female (women 55% vs men 37%, p<0.0001), higher education level (tertiary 58% vs less than tertiary 38%, p<0.0001), and having quit smoking (past smokers 52% vs current smokers 41%, p=0.005).

Conclusions: This is the largest known assessment of CAM use in individuals at high risk for breast cancer. The prevalence and predictors of CAM use in this cohort of individuals at high risk for cancer is similar to that reported in the general Australian population. Only a small proportion were using CAM specifically to prevent cancer.
Magnetic resonance imaging (MRI) in conjunction with mammography has been shown to increase the sensitivity of breast cancer detection in individuals at high risk of developing breast cancer based on genetic test results or family history. In our region, surgeons have been ordering MRI’s for their high risk patients for approximately 2 years. In 2007 dedicated funding for 40 MRI’s for this at-risk group was obtained. Two breast cancers have been detected by MRI that were not evident on mammogram. We present our experience of the implementation of this service and review international guidelines for high risk breast surveillance using MRI.
FAMILY HISTORY AND THE COST EFFECTIVENESS OF SCREENING FOR COLORECTAL CANCER

K Rigby [1], J Litt [1], D Roder [2], G Young [3], G Suthers [4].


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We examined the cost effectiveness of screening for colorectal cancer (CRC) among cohorts with and without family histories of CRC. The population had 98% at standard risk, 1.5% at moderate risk (4.5-fold RR), and 0.5% at high risk (15-fold RR); genetic testing for heritable mutations was incorporated for individuals at increased risk.

The point cost effectiveness ratio for the aggregate of all age/risk groups was $US5,660 per DALY averted. The ratio varied five-fold across the disaggregated groups, with screening of older increased-risk subjects being more cost effective than screening of either older standard-risk or younger increased-risk subjects. Incremental cost effectiveness varied four-fold among different age/risk categories, with surveillance of high risk (at any age) and moderate risk (aged over 50) being the most cost effective. In contrast to previous CRC economic models, health professional adherence to guidelines was responsible for more variation than patient adherence.

We conclude that aggregated data that assumes a homogeneous risk profile masks the cost effectiveness of different age/risk categories. Additional resources for CRC screening would be best directed to individuals with a family history of CRC. The cost effectiveness of CRC screening programmes, irrespective of family history, are more dependent on the adherence of clinicians to guidelines than the adherence of patients to the clinician’s recommendations.
SAMPLE REQUIREMENTS FOR MEDICAL GENETIC TESTING: DO GENETIC TESTS DEMAND A DIFFERENT STANDARD?

G Suthers [1], D Ravine [2].


E-mail: graeme.suthers@cywhs.sa.gov.au

Approximately 0.15% of sample tubes have blood from the wrong patient. The significance of sampling errors varies according to the probability of the erroneous result being clinically relevant and the availability of corroborating evidence. Errors in genetic testing are of particular concern because there may be little or no corroborating evidence. A survey of Australasian laboratories in 2005 identified different sampling protocols that varied within and between laboratories.

It is suggested that

1. a single sample is appropriate for tests that carry few implications for relatives eg tests for somatic variants, population-based carrier testing;
2. duplicate sampling and testing (or comparable corroborations) be implemented for tests which carry major implications for patient and relatives eg presymptomatic tests; and
3. a single signed specimen of foetal samples be collected for prenatal testing.

The introduction of duplicate sampling results in costs of a few hundred thousand dollars per erroneous result avoided. Such costs need to be set against the cost of a successful medical indemnity claim, particularly one involving multiple family members.

The clinical significance of a sampling error varies with the clinical context, and the pathology laboratory will not necessarily be aware of this context. Hence the decision to utilise different sampling protocols to reduce the risk of sample errors rests with the clinician requesting the test.
BRCA1/2 PREDICTIVE TESTING: WHY DO WE HAVE A LOW NUMBER OF PREDICTIVE TESTS PER PROBAND?

Koehler J, Tyler J, Tucker K, Andrews L & Kotchetkova I
Hereditary Cancer Clinic, Prince of Wales Hospital.

Background: Once a BRCA1/2 mutation is identified, at-risk relatives can have their cancer risk clarified by predictive testing. A number of studies have tried to quantify the uptake of predictive testing in these families with varying results. The Prince of Wales Hereditary Cancer Clinic has a large database of mutation positive families dating back to 1995, which would be suitable to study uptake of BRCA1/2 predictive testing.

Aims: To investigate the uptake of predictive testing in BRCA1/2 families known to the Prince of Wales Hospital Hereditary Cancer Clinic and associated services.

Method: Families were a BRCA1/2 mutation had been identified at least one year prior were retrieved from the clinic database. The number of predictive tests, both positive and negative, in each of these families was recorded. Data from families that were seen for predictive testing only (mutation identified at another clinic) was also retrieved.

Results: For the 155 families where the proband was identified by the POW service, on average 1.4 predictive tests per proband were performed (range 0-18). A significant number of probands had no other family members tested at our service. One explanation for the seemingly low number of predictive tests is that eligible relatives may be accessing other genetics services for predictive testing. To attempt to account for this bias, the average was adjusted to include additional 94 families who attended the POW service for predictive testing only (mutation identified elsewhere). The adjusted average of predictive tests per proband was 2.1.

Discussion: It appears that overall the uptake of predictive testing in our families is low. There are a number of possible explanations for this, however it is not possible to identify these from the database alone.

Outcomes: To further clarify uptake of BRCA1/2 testing by eligible individuals we have decided to systematically review the POW BRCA1/2 families by telephone. The review is focusing on dissemination of information and uptake of genetic testing in families however it is also being used to check psychosocial coping, current breast/ovarian cancer risk management strategies, and suitability for current research projects of mutation carriers.

The review is currently in progress. Due to the large number of mutation positive families it is likely to take some time to complete. Preliminary findings will be presented.
Over the past three years, Genetic Technologies Ltd (GTG) has been providing a fee for service BRCA 1 & 2 genetic pathology testing service. Full gene screening is performed within 8 weeks and fast tracking of testing for clinical management purposes can be performed within 3 weeks.

We present a schema for testing that utilizes robotics, LIMS, automated DNA sequencing, computer aided analysis and ISO15189 / NATA / RCPA accredited test protocols.

Despite the active testing that has been carried out of the BRCA1 & 2 genes worldwide over the past decade, it is interesting to note that significant numbers of new (not previously reported) BRCA mutational events and gene variants have been identified in our testing service - the GTG experience.
CANCER, CULTURE AND GENETICS

Mona Saleh\textsuperscript{1,2}, Bettina Meiser\textsuperscript{2}, Kathy Tucker\textsuperscript{3}, Judy Kirk\textsuperscript{4} & Kristine Barlow-Stewart\textsuperscript{1}

1 - The Centre for Genetics Education, NSW Health, NSW
2 - Psychosocial Research Group, Prince of Wales Hospital, NSW
3 - Hereditary Cancer Clinic, Prince of Wales Hospital, NSW
4 - Familial Cancer Service, Westmead Hospital, NSW

Building on previous research in the Chinese-Australian community, our aim is to explore how individuals’ cultural backgrounds shape their beliefs about genetics, kinship, inheritance and cancer. Using ethnography, Arabic and Anglo/Celtic Australians will be asked about their experiences assimilating information about inherited cancer susceptibility into their personal culture.

Group 1 will consist of approximately 15 individuals who identify themselves as an Arabic-Australian and they or a member of their immediate family speaks Arabic or has a country of birth or ancestry from a predominantly Arabic speaking country. These individuals will have previously attended a hereditary cancer clinic in NSW through which they will be recruited.

Group 2 will consist of approximately 15 individuals who identify themselves as an Anglo/Celtic-Australian and they, their parents and all grandparents have a country of birth from a predominantly English speaking country. These individuals will have previously attended a hereditary cancer clinic in NSW through which they will be recruited.

We present the significance of using ethnography in this study enabling a comparison of the data from the Arabic, Chinese and Anglo/Celtic Australians. This comparison will identify any common ground or significant differences in the way information about hereditary cancer is processed by these culturally distinct groups.
PSYCHOLOGICAL RESPONSES TO GENETIC RISK INFORMATION AMONG INDIVIDUALS WITH A STRONG FAMILY HISTORY OF MELANOMA: PRELIMINARY FINDINGS

Graham Mann1, Nadine Kasparian2,3, Bettina Meiser2,3, Phyllis Butow4 & Judy Simpson5.

1 Westmead Institute for Cancer Research, The University of Sydney at Westmead Millennium Institute.
2 Psychosocial Research Group, Department of Medical Oncology, Prince of Wales Hospital.
3 School of Psychiatry, Faculty of Medicine, University of New South Wales.
4 Medical Psychology Research Unit, School of Psychology, The University of Sydney.
5 School of Public Health, The University of Sydney.

Purpose: Limited data are currently available on the psychological experiences of individuals with a strong family history of melanoma. This prospective cohort study examined uptake of genetic testing for melanoma risk and psychological adjustment to genetic test results in a sample of high-risk families with identified family-specific mutations.

Methodology: Individuals with a strong family history of melanoma (i.e. families comprising > 2 relatives with a confirmed melanoma diagnosis) were ascertained via the Westmead Institute for Cancer Research/University of Sydney centre of the Genetic Epidemiology of Melanoma Study. Individuals were eligible for participation if a family-specific mutation in the CDKN2A gene had been identified via the research protocol. Ineligibility criteria included previous genetic testing for melanoma risk, current metastatic cancer, or limited English literacy skills. Using a series of mailed, self-report questionnaires, data were collected at two time-points: notification of genetic testing availability (January 2005), and either two weeks after receipt of genetic test results (for testees), or 12 months after initial notification (for test decliners).

Results: One-hundred sixty-eight eligible individuals were approached for study participation. Of these, 121 (48% male) returned baseline questionnaires, yielding a response rate of 72%. At baseline, mean psychological distress scores were relatively low. Participants previously affected with cancer exhibited significantly higher levels of melanoma-specific distress than unaffected (Z=-3.18; p=0.001), but equivalent values of generalized anxiety (p=0.25) and depression (p=0.23). Having a personal history of melanoma (OR=3.37, p=0.033), perceiving greater family implications of melanoma (OR=2.52, p<0.0001), and the tendency to monitor for risk-related information (OR=3.12, p=0.008) were associated with melanoma-specific distress. Not having any children (β=2.09, p=0.007), perceiving sun exposure as an important cause of melanoma (β=1.15, p=0.015), and perceiving greater family implications of melanoma (β=1.02, p=0.002) were associated with generalized anxiety. As of July 2007, 17 (14%) participants have undergone genetic testing, with 81% identified as mutation carriers. Preliminary data show that irrespective of result, testees reported significantly higher levels of melanoma-specific distress compared to decliners at baseline (Z=-2.27 p=0.02). Compared to baseline, carriers reported significantly reduced depression scores 2 weeks after receipt of a positive result (Z=-2.25, p=0.024).

Conclusion: As in other common familial cancers, distress was relatively uncommon in this familial melanoma cohort, even after notification of the presence of a family mutation and receipt of positive test results. It is pertinent to examine low levels of genetic testing uptake, as well as psychological responses to testing, in the context of behavioral intentions.
PERSONAL THEORIES OF INHERITANCE IN GENETIC COUNSELLING

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South Coast Mail Centre
NSW 2521
Ph 02 4222 5576

McAllister’s model of Personal Theories of Inheritance (2003) was developed from a grounded theory study to explain variations in adjustment to genetic testing for Hereditary Non-Polyposis Colon Cancer, in which participants at risk of HNPCC, considering genetic testing, developed personal theories of inheritance (PTIs) that were perceived by the clients as assisting in coping and decision making.

Five cases of persons at risk of familial cancer derived from clinical practice are presented. These cases are examined with respect to the individual Personal Theories of Inheritance developed by the probands, with particular attention paid to the development of the PTI and relationship to significant life events, and the role of the PTI in assisting coping.

These cases are followed by a brief discussion about possible counselling skills and strategies that may be useful, including:

- a review of the role of exploration of the PTI with the client (when it is helpful and when it may not be),
- useful aspects of the PTI; its use in client empowerment and decision making
- PTIs and incorrect information; what could or should be done?

In addition, the possibility of an irrational and possible harmful PTI is reviewed, with suggestions for genetic counselling practice drawn from the psychological concept of parallel processing.
INFORMATION & SUPPORT GROUPS FOR FEMALE BRCA CARRIERS

Sally Russell [1], Vanessa Huntley [1], Jacqueline Armstrong [1], Debra Trott [1], Graeme Suthers [1], Nicola Poplawski [1].
[1]Familial Cancer Unit, Women’s & Children’s Hospital, North Adelaide SA 5006
Email: sally.russell@cywhs.sa.gov.au

Background
The development of a group program was initiated following results from a client survey in 2004 which indicated a need for extra information and support amongst the survey’s respondents.
Whilst two of these groups have been reported on at a previous KConfab meeting, we have now completed the third in this pilot series and feel that value can be derived from reporting on the outcomes, and highlighting salient points that have emerged for future practice.

Composition of groups
Group 1- Comprised 15 BRCA carriers who had not had a cancer diagnosis.
Group 2- Comprised 9 BRCA carriers, all had been diagnosed with cancer (8 breast and 1 ovarian).
Group 3- Comprised 21 BRCA carriers, 14 diagnosed with cancer and 8 not having had cancer.
Only female participants were invited, and these women represented a wide range of ages and length of time since BRCA status diagnosed; ranged from 9 years to 3 months.

Program
All three groups have followed the same structure of one 2 hour session held once a month over 5 months. Over the course of the 3 groups we changed the program to incorporate suggestions made by the women in their evaluation forms. Interestingly the changes resulted in less emphasis on issues about communicating with family, coping and stress management, to more emphasis on practical information from expert speakers such as a gynaecologist and breast surgeon.

Benefits for participants
A number of benefits have been identified by the women either in verbal discussion with the group leaders or in the evaluation forms they filled in after each session. These include:
• meeting other women ‘in the same boat’
• clarifying information around prophylactic surgery and thus feeling able to make informed decisions about, and take action in relation to, prophylactic surgery
• getting detailed information about areas that may not have been discussed at the time of their genetic testing (or may not have been available) e.g. reproductive options

Future Directions
The SA Familial Cancer Unit intends to offer these groups to all women newly diagnosed with a BRCA mutation as well as to those women who have been previously invited but not attended a group. We believe this opportunity is a worthwhile adjunct to the testing process in providing additional support and information to women with a BRCA mutation.

“Familial Cancer 2007: Research and Practice”
PROTOCOL AND PRELIMINARY DATA FROM A HIGH RISK BREAST CANCER SCREENING CLINIC

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Introduction
In Australia, government breast screening programs do not address the needs of young high-risk patients. In 2004, at Royal North Shore we established a high-risk multidisciplinary screening clinic. Risk assessment, regular clinical and radiological review and risk reducing strategies are offered.

Aim
This study aims to present the screening protocols for this group and audit the preliminary results comparing them to routine and high-risk screening programs nationally and internationally.

Methodology
Data was collected prospectively from 60 patients categorized as high risk based on family history or mutation status at biannual examinations.

Results
The mean age was 41 years. 29% were from mutation positive families, 32% had non-informative family testing. 32% had a family history of ovarian cancer, 3% of male breast cancer and 7% of Ashkenazi ancestry. 69% had children. 25% had used the oral contraceptive pill, 10% hormone therapy and 2% infertility treatment.

The call back rate was 24% on first round screening and 11% on subsequent rounds. 10% had prophylactic surgery. One patient who had bilateral mastectomy and tram flap reconstruction has developed pancreatic cancer.

Conclusion
The call back rate for this group of patients is much greater than that of the routine screening population due to a higher index of suspicion and lower interventional threshold. As the clinic matures, this rate will potentially decrease. The sensitivity of screening is anticipated to improve with the addition of MRI.
EVALUATION OF A RISK MANAGEMENT CLINIC FOR WOMEN AT HIGH GENETIC RISK OF BREAST AND OVARIAN CANCER.

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A Risk Management Clinic (RMC) providing multidisciplinary care for women at high risk of breast and ovarian cancer was established in 2006 by the Familial Cancer Service at Westmead Hospital. This service has identified approximately 200 women with germline BRCA1 or BRCA2 mutations. These women and some others with a strong family history of breast/ovarian cancer were invited to attend the RMC. Details of eligibility criteria for invitation to attend and reasons for non-attendance have been previously presented.¹

The RMC is staffed by a cancer geneticist, a breast physician, a gynaecological oncologist and a clinical nurse consultant. Women undergo screening for breast and ovarian cancer and are provided with detailed information and advice about risk-reducing strategies including prophylactic surgery.

Thirty-six women attended the monthly clinics during its first year in 2006. The average age of clinic attendees was 42 years (range 25 — 67). This included 16 BRCA1 gene mutation carriers, 14 BRCA2 gene mutation carriers and six women with a potentially high-risk family history with an inconclusive or unavailable result from genetic testing. Twenty-nine were unaffected by cancer, six had previously been diagnosed with breast cancer and one with ovarian cancer.

A questionnaire was mailed to the 36 participants after they attended the clinic, seeking their feedback on the clinic and the services offered. The questionnaire sought information about reasons for attendance and satisfaction with various aspects of the clinic. Twenty-six women returned completed questionnaires (response rate 72%).

Respondents expressed a high level of overall satisfaction with the clinic with 96% stating they were satisfied or very satisfied with their experience. The most frequent reason for attending the clinic was to have screening tests performed (45%). All of the respondents felt that the mix of clinical experts providing care was useful and all expressed an intention to return the following year. Eighty-five per cent thought that the planned annual visit was appropriate and the remaining 15% stated that they would rather return at six-monthly intervals.

Uptake of risk reducing surgery was high with 71% of gene mutation carriers of appropriate age and unaffected by cancer undergoing bilateral salpingo-oophorectomy. One woman was diagnosed with breast cancer and none with ovarian cancer during the first year of RMC.

The evaluation has provided evidence that the RMC, in its current form, is meeting many of the needs of women at high genetic risk of breast and ovarian cancer.

Acknowledgments
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The authors acknowledge the staff at the Peter MacCallum Cancer Institute Risk Management Clinic and the Royal Marsden Hospital for their assistance in the development of the clinic and the evaluation.

Reference
EVALUATING THE EFFECTIVENESS OF COMMUNICATION SKILLS TRAINING FOR GENETIC COUNSELLING

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Pam McLean Cancer Communications Centre and the Medical Research Psychology Unit, University of Sydney, in conjunction with the Centre for Genetics Education (CGE) NSW Health have been providing one-day communication skills training workshops for genetic counselling since 2002. Anecdotally, the genetic counsellors report great value from these workshops, but providers such as CGE, need real evidence that they make a difference to counsellor confidence and practice. It is difficult to find evidence in this area in the literature. An outcome evaluation of the communication skills workshops was undertaken to determine if there was any self reported change in genetic counselling practice as a result.

Genetic counsellors and geneticists (42) who have attended one of the three CGE communication skills training workshops conducted in 2002, 2004 and 2006, were asked to complete a questionnaire concerning their experience of the workshop, what they learned, whether and how they have implemented that learning, what they would seek from future workshops and their self-perceived level of stress in their work. The questionnaire was developed through consultation with experienced genetic counsellors and includes the Maslach Burnout Inventory. Evaluation also covered short term outcomes recorded immediately post workshops and a detailed description of the process of developing case scenarios, running the workshop and some of the strategies that emerged during the process.

This paper reports on the findings of this study and recommendations for communications skills training in this format.
STARTING THE CONVERSATION – AN EVALUATION OF THE NSW FAMILY HEALTH HISTORY CAMPAIGN.

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Internationally campaigns have been conducted to promote correct reporting of family health history (FHH) by patients. The NSW Health community FHH campaign, August 2006, encouraged discussion by patients with their family and taking the recorded information to their doctor. Conditions targeted were diabetes type 2, heart disease and breast, ovarian, bowel and prostate cancer where there are clinical guidelines related to family health history in place.

Development involved community poll surveys, consumer and GP consultations and informing and resourcing GPs State-wide. The limited, small budget media campaign targeting the community included print, radio and a television interview on Australia’s most popular breakfast show. Post campaign evaluation strategies included: survey of GPs (2 NSW Divisions: 606 GPs); Poll survey (400 community members) and analysis of enquiries, website and media coverage.

28% (112) of the community randomly polled had seen or heard of the campaign; of these 48% reported discussing FHH with family members as a result; 35% had initiated a discussion with their doctor about their FHH. Website activity, while low, peaked immediately post the campaign (August: 324 visits) with particular interest in the FHH Record.

30% of GPs that returned the mailed survey had heard of the campaign; of these 66% reported an increase in the number of patients who told them about their family history since the campaign. GPs reported more confidence about information given by patients, the simplicity of the FHH record and some had made referrals as a result.

While awareness of the campaign itself was limited, for those who were aware, the message had a significant impact. Benefit may also be seen where both the patient and the GP are informed. The findings suggest that there are good grounds for moving towards a national FHH campaign.

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MAKING THE COMPLEX SIMPLE: USING AN ON-LINE TOOL TO ESTIMATE CANCER RISK FOR WOMEN WITH A FAMILY HISTORY OF BREAST OR OVARIAN CANCER

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Information about a woman’s family history of breast and ovarian cancer is an important aspect of clinical management, following discovery of the BRCA1 and BRCA2 genes, in which germline mutations are associated with breast and ovarian cancer predisposition. A strong genetic predisposition accounts for only around 5-10% of cases of breast and ovarian cancer but many women are concerned about their family history.

Both general practitioners and specialists see women who are currently asymptomatic but have raised concerns about familial aspects of cancer, or women who have had a diagnosis of cancer and are anxious about the implications of their diagnosis on other family members. The determinants of familial risk are complex, and familial risk is difficult to estimate, so health professionals need assistance to facilitate discussions about risk with their patients.

In 2006 the National Breast Cancer Centre (NBCC) revised and released \textit{Familial aspects of breast cancer and epithelial ovarian cancer: a guide for health professionals}. This guide has now been used as the basis for the development of an on-line decision tool. With a maximum of eight questions required, the on-line tool enables the collection of an accurate family cancer history, for both maternal and paternal sides of a woman’s family, covering a large number of scenarios. As part of the development of the on-line tool, it was necessary to address a number of family history scenarios that are not specifically covered by the current published guidelines.

Data about key factors associated with increased risk such as the number of relatives affected by breast and ovarian cancer and age at diagnosis are gathered using a simple, on-screen format.

The on-line tool about familial aspects of breast and ovarian cancer will address a need for appropriate, readily accessible, and evidence-based information to support the consultation between women and their clinicians, and to determine appropriate screening and/or interventions. The on-line tool will be piloted in a range of clinical settings before being made available to health professionals via the NBCC web site.
A BALANCING ACT - TELEHEALTH CANCER GENETICS AND CLINICIANS' EXPERIENCES OF A TRIADIC CONSULTATION.

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Background: With increasing demand for genetic services, telehealth has become a viable alternative to face-to-face consultations. Telegenetics involves the interactions of three parties: geneticist, genetic counsellor and client, creating a triadic consultation, where one practitioner gains access through remote technology.

Objective: The aim of this study was to explore clinical geneticist's and genetic counsellor's perspectives and experiences with telegenetics, and their changing roles and expectations within the consultation.

Methods: Semi-structured interviews were conducted with practitioners delivering telegenetics services in New South Wales. The interviews explored experiences of telegenetics, satisfaction, perceived aims of the service, advantages and disadvantages of the technology, and roles within the consultation. Interviews were transcribed and thematically analysed.

Results: Six geneticists and nine genetic counsellors were interviewed. Practitioners express a high level of satisfaction with telehealth services. They saw it as an efficient means of offering equitable specialist services to rural and outreach areas. They also felt that patients tended to quickly feel comfortable with the medium, and appreciated telehealth in general, and in particular the fact that it made redundant the need to travel to a major centre. They also expressed the view that telehealth equipment was generally reliable and easy to use and offered adequate visual resolution. Throughout the telehealth process, genetic counsellors reported taking on many roles, including that of facilitator, administrative assistant and counsellor. They reported that they identified with both the client and the geneticist, and changed their target of identification throughout the consultation. In telegenetics the role of the geneticist may become one of the consulting specialist taking on a leadership role during the consultation, delivering medical information and screening advice. As geneticists were not physically present with the client, they saw themselves as depending on the genetic counsellor to be their "eyes, ears and hands", and believed telehealth ran more smoothly when they had confidence in the genetic counsellor's experience and skills.

Practitioners reported that interactions in telehealth are moderated by physical positioning of the genetic counsellor and client, and whether the counsellor appears onscreen. This had the potential to reduce the interaction to a more traditional medically modelled doctor-patient interaction. Whilst this communication process is more focused, it may ignore interactions occurring off screen. The challenges and benefits of each approach will be presented and discussed.

Conclusions: Practitioners are highly satisfied delivering genetic services through telehealth however they acknowledge the trade off involved in offering remote consultations.
AN OVERVIEW OF THE KCONFAB BIOSPECIMEN BANK: 10 YEARS ON

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KConFab is the Australian – New Zealand research consortium for research into families at high risk of breast and ovarian cancer. To date, 1182 families have been recruited. Genetic, epidemiological, medical and psychosocial data along with biological samples are collected from affected and unaffected, female and male participants over the age of 18. This material is available to researchers worldwide that have obtained peer reviewed, ethically approved and funded research projects. At present, kConFab is supplying biological specimens and data to more than 50 research projects.

The kConFab biological repository contains bloods from 10353 participants, this represents 8.8 bloods collected on average from each family. The standardized kConFab blood processing protocol produces fractions of plasma, non lymph, blood pellet and white blood cells separated using a ficol gradient. White blood cells undergo EBV transformation to produce cell lines which are used by researchers in functional assay or as a replacement source of dna/rna for the bank. As of May 2006, EBV cell line transformations have been performed on 720 participants. Along with a blood sample participants complete epidemiological questionaries, to date 10244 questionaries have been completed.

The kConFab Tissue bank was established in 1997, and currently averaging four tissue collections per week. The kConFab tissue bank is composed of 57% breast tissue, 31% ovarian tissue, with the remaining 12% comprising other tissues such as bowel, spleen and brain. A large proportion of prophylactic mastectomy and oophorectomy specimens are collected on a regular basis as many mutation positive participants opt for this surgery. Tumour tissue collections are a very valuable source of the kConFab bank. In most cases tumour tissue collections are matched with an aliquot of normal tissue from a distal area that has clearly not been affected with tumour. Consequently, tumour tissue amounts to 25% of the bank, while normal tissue comprises 75%.

A full research pathology review is conducted on the tissue collected by our pathologist using standardised scoresheet for normal and tumour tissues. Important features such as percentage tumour, normal epithelial, lymph and necrotic components are scored.
Genetic testing for familial cancer predisposition has the potential to identify family specific mutations, variants of unknown clinical significance and polymorphisms. The Central & Southern Regional Genetic Service has created a results database for cancer genetic testing to keep track of all of the identified cancer gene alterations.

This database enables clinicians to search on a specific gene alteration and identify families who have same alteration. This project was initiated following concerns about pathogenicity of certain gene mutations and the methods of recall in place for these families. Since New Zealand is a relatively small population, this ongoing project endeavours to trace all cancer predisposition gene alterations identified to link families, track variants and possibly identify founder mutations. To date, approximately 260 patients have been entered retrospectively from 155 families who have had BRCA testing. There are three families who have been identified to have a common mutation. Alterations identified in bowel cancer predisposition genes are currently being entered. This presentation will highlight the features of the database along with examples of how it can be used in clinical practice.
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AUSTRALIAN FAMILY CANCER REGISTRIES - A COLLABORATIVE WALKABOUT

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Australia is a federal country in which health issues are the responsibility of each individual state and territory. Six family cancer registers operate, each serving the populations of their state or territory and collectively they have 4015 consented registrants. There is however wide variation in the populations and geographic dispersal of relevant health and genetic services in each state, and each register has had its own historical development pathway. All the registers began as FAP registers and evolved into family bowel registers to include HNPCC and other high-risk bowel cancer syndromes. Currently there is a changing trend for the family bowel cancer registers to evolve into family cancer registers addressing other identified familial cancer syndromes.

The registers are managed independently of each other but maintain contact by sharing knowledge and information. However, the people, the diseases, the health systems and the knowledge-base are comparable and there is therefore considerable similarity in the objectives and structures of the different registers. Their attributes and differences are described in this poster.
Familial Cancer 2007: Research and Practice

SEBACEOUS ADENOMAS AND CARCINOMAS- IS THIS MUIR-TORRE SYNDROME? : ONE CLINIC’S RECENT EXPERIENCES

Poonam Zodgekar, Georgina Fenton, Annabel Goodwin, Judy Kirk

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We describe a series of cases where patients have presented with sebaceous adenomas or carcinomas that showed loss of staining for the mismatch repair proteins, in the absence of any firm associated family history suggestive of Hereditary Non-Polyposis Colorectal Cancer (HNPCC).

Case 1: CM presented to the Familial Cancer Service in 2005. CM’s father, DM, had been given a diagnosis of Muir-Torre Syndrome (MTS) after being diagnosed with a sebaceous carcinoma, which showed loss of staining for hMSH2 and hMSH6 (in Queensland). DM then had a colonoscopy that detected a rectal tubulovillous adenoma. Immunohistochemical staining on this adenoma also showed loss of hMSH2 and hMSH6. At this stage, a clinical diagnosis of MTS was made, and DM was referred to Queensland Genetics for consideration of genetic testing. Testing of hMSH2 has been inconclusive (June 2007). Screening as per HNPCC has been advised for close family members.

Case 2: SP, a 69-year old gentleman with chronic renal failure on peritoneal dialysis was diagnosed with a sebaceous carcinoma that showed loss of staining for hMSH2. There was no family history of HNPCC-related malignancies but limited family history was known. SP was advised to have a colonoscopy. This was unlikely to be MTS if there were no polyps or malignancy detected. SP chose not to proceed with colonoscopy due to concerns about potential complications that may interfere with his peritoneal dialysis. Following our consultation, blood was taken for genetic testing. Genetic testing was inconclusive (July 2007). As SP has not had a colonoscopy, moderate risk screening with 3-5 yearly colonoscopies was advised for his offspring.

Case 3: JG, a 60-year old man, presented to the Familial Cancer Service, after being diagnosed with a sebaceous carcinoma, which showed loss of staining for hMLH1 and PMS2. At colonoscopy, JG was found to have 3 adenomatous polyps. Immunohistochemistry was performed on one of these polyps and was normal. JG’s mother had been diagnosed with a transitional cell cancer of the bladder aged 70. There was no other family history of HNPCC-related cancers. Genetic testing was commenced on JG. Results of testing on hMLH1 have been inconclusive (July 2007). JG has developed further tiny adenomatous polyps and hence a repeat colonoscopy has been advised in 2 years. Close family members are advised to have colonoscopies from 40 years, and then every 3-5 years as for those at moderate risk of bowel cancer.

Case 4: A 65-year old woman, BG, presented after being diagnosed with a sebaceous carcinoma. Immunohistochemical staining showed loss of hMSH2. The accuracy of this staining was uncertain. However, repeat staining on the tumour sample confirmed loss of hMSH2 and hMSH6. Blood was taken for genetic testing. After a difficult colonoscopy, she was found to have a tubular adenoma. There is no family history of HNPCC-related malignancies.

Case 5: A 66-year old lady, YC, presented with a history of multiple skin tumours, including a sebaceous adenoma, an acanthoma and a sebaceous carcinoma diagnosed over a period of 20 years. The most recent sebaceous carcinoma showed loss of staining for hMSH2 and hMSH6, prompting referral. Investigation of the family history revealed that YC’s brother had been found to have some tubular adenomas. YC is awaiting colonoscopy and meanwhile, genetic testing has proceeded.

This case series of patients diagnosed with skin lesions associated with MTS, a variant of HNPCC, but no family history of HNPCC related internal malignancies is unusual. All patients presented due to a diagnosis of sebaceous carcinoma and loss of staining for the mismatch repair proteins in the tumour sample. Further investigations may help determine whether this is indeed Muir-Torre Syndrome.

“Familial Cancer 2007: Research and Practice”
STABILITY OF BAT26 IN MSH2-DEFICIENT LYNCH SYNDROME COLORECTAL TUMOURS

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Tumours showing instability in 30–40% or more of microsatellite markers are designated MSI-H, and this phenotype is tightly associated with Lynch Syndrome in early onset cancers. One microsatellite marker, BAT26, has shown particularly high sensitivity and specificity in the detection of an MSI-H phenotype in colorectal tumours, and it has been suggested that this marker alone could be used to screen for Lynch Syndrome. BAT26 is located immediately downstream of exon 5 in the MSH2 gene, and consists of a poly-A repeat of an invariant number of nucleotides which is particularly susceptible to deletion-type replication errors in states of MMR deficiency. Recently, it has been demonstrated that in individuals with large germline deletions of MSH2 which span exon 5, BAT26 may show no MSI when tested on tumour tissue. Therefore, we investigated the stability of BAT26 in a series of 92 confirmed germline MMR mutation carriers. Of these 92 cases, 51 carried mutations in MSH2, and 41 in MLH1. All cases had undergone tumour MSI analysis and immunohistochemistry for MMR genes. Twelve of 51 (24%) MSH2 mutation carriers were found to have large exonic deletions, 7 of which spanned exon 5. Of these 7, 4 showed stability at BAT26 in their colorectal tumours. In the remaining 44 cases, where exon 5 was not deleted, only 5 were stable at BAT26. Therefore, the rate of BAT26 stability in cases with large deletions spanning exon 5 (4/7 or 57%) was significantly higher than in both all other types of MSH2 mutation (5/44 or 11%)(p=0.014, Fishers Exact Test), and in MLH1 mutation carriers (0/41). This work suggests that the finding of BAT26 stability in an MSH2-deficient tumour may indicate a large deletion encompassing exon 5. In addition, the results highlight a limitation of using BAT26 alone for the detection of MMR deficiency.
Endometrial cancer is the most common invasive gynaecological cancer in Australia. Several histological subtypes are recognised, with poor survival for some, but few epidemiological studies have distinguished between different types. Also, little is known regarding the role of germline genetic factors in endometrial cancer risk, except that an increased cancer risk exists among relatives. In order to establish an integrated approach to endometrial cancer research, we have initiated collection of epidemiological and clinical data and biological samples from a nationwide population-based group of more than 1000 women newly diagnosed with endometrial cancer, a comparable group of cancer-free women, and approximately 400 selected relatives of cases reporting a family history of cancer. The specific aims of the Australian National Endometrial Cancer Study are to: clarify existing and identify new modifiable risk factors, and their interaction with genetic factors, by subtype; examine the genetic basis to disease within multiple-case families; establish and maintain a biorepository and epidemiological, molecular and clinical database for ongoing studies. A network of more than 40 collaborating clinicians has been established for case recruitment and provision of pathology expertise, and the study is currently recruiting women with endometrial cancer via collaborating clinicians at hospitals, population controls identified via the electoral roll and other sources, and relatives of cases reporting a family history of cancer. Participants provide information on risk factors, dietary habits, and if possible, a blood sample. Patients consent to collection of a tumour tissue sample and access to medical records. Preliminary analysis of reported family history of cancer in cases compared to controls revealed that cases report more family history of any cancer, irrespective of degree of relationship, or number of affected relatives considered. Differences were statistically significant for reports of 1st and 2nd degree family history of endometrial cancer alone, colorectal and/or endometrial cancer, and breast/ovarian/endometrial cancer. A subset of cases is being screened for tumour pathology features suggestive of mismatch repair (MMR) gene mutations, and DNA of selected cases is being screened for mutations in the MMR and PTEN genes. Cases with proven or possible MMR gene mutations (as indicated by loss of MMR protein expression) report a variable extent of family history, with unremarkable or unusual family history of cancer for a subset. Our results suggest that MMR gene mutations detected in population-based cases may be associated with family history profiles less distinct then those generally used in the genetic counselling clinics.
IDENTIFYING PMS2 FAMILIES IN A FAMILIAL CANCER SETTING: THE ROLE OF IMMUNOHISTOCHEMISTRY TESTING

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It is well established that germline mutations in the mismatch repair genes, MLH1, MSH2, and MSH6 cause hereditary nonpolyposis colorectal cancer (HNPCC). More recently germline mutations in another mismatch repair gene, PMS2, have also been shown to be associated with HNPCC, although the relative contribution of these mutations to the epidemiology of HNPCC remains uncertain. To date few families with germline PMS2 mutations have been described in the literature.

Aim:
The aim of this presentation is to describe the phenotype of 4 families who harbour a PMS2 mutation, and demonstrate the role of immunohistochemistry (IHC) testing in identifying these families in a Familial Cancer Clinic setting.

Findings/Outcomes:
• IHC testing was performed on colorectal or endometrial cancer tissue. Results showed loss of PMS2 expression alone, or concurrently with MLH1 deficiency.
• The PMS2 mutations were detected through a staged process, whereby MLH1 mutation analysis proved negative in the first instance.
• Two of the families fulfilled the Amsterdam criteria II for HNPCC, 3 of the probands had a colorectal or endometrial cancer diagnosis under the age 50.

Conclusion:
• Our clinic experience supports the use of IHC in identifying families with PMS2 mutations, and suggest PMS2 staining should be routinely used in the IHC panel for the mismatch repair proteins
• PMS2 mutation analysis is indicated in patients showing absent staining for PMS2 protein, and it is possible to consider proceeding with PMS2 mutation analysis before MLH1 as a cost saving strategy, when there is exclusive PMS2 loss of expression
• Concurrent MLH1 and PMS2 absence should not be used as an exclusion criteria for mutation analysis of PMS2, when MLH1 mutation detection proves negative
• Our clinic experience supports the possibility that PMS2 mutations may be more frequent than originally expected.
Hereditary Non Polyposis Colon Cancer (HNPCC) is the cause of about 8% of all colorectal cancer. The hallmark of HNPCC is defects in DNA mismatch repair leading to microsatellite instability (MSI). At the molecular level, HNPCC is caused by mutations in the mismatch repair genes (MSH2, MSH6, MLH1 and PMS2). These genes perform their biological function via MSH2/MSH6 or MLH1/PMS2 heterodimers. The great majority of HNPCC reported to date has been due to a mutation in either the MSH2 or MLH1 gene and only rarely due to a mutation in the MSH6 or PMS2 genes. The detection of mutations in the PMS2 gene in the past has been hampered by the presence of numerous pseudogenes however this problem has been overcome by selecting primers that bind to regions of DNA unique to the ‘true’ PMS2 gene. While the majority of mutations reported can be attributed to pathogenic sequence variation, like many other genes, exon deletions and duplications have been reported and can be readily detected by Multiplex Ligation-dependent Probe Amplification (MLPA).

We have used both direct sequencing and MLPA to detect mutations in the PMS2 gene. To date we have performed PMS2 genetic testing on a cohort of 51 patients from around Australia and overseas, all with appropriate informed consent. In the main these patients had: a loss of both MLH1 and PMS2 proteins by IHC, a loss of PMS2 only or in some cases we had no IHC data. Of these 51 patients, 9 patients were found to harbour sequence variations that were considered pathogenic (1 truncating mutation, 4 indels, 1 small deletion, 1 single nucleotide insertion and 2 missense mutations). In addition, we detected 3 with missense sequence variants of unknown clinical significance. Using MLPA we have identified an additional 7 mutations (2 whole gene deletions, 4 with a deletion of exon 10 and 1 with a deletion of exon 7).

In conclusion, we have detected a pathogenic mutation in 16 patients, a mutation detection rate of approximately 31%.
PAPILLARY RENAL CELL CANCER - IDENTIFICATION OF A DOMINANTLY INHERITED MET PROTO-ONCOGENE MUTATION

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Background: Papillary renal cell carcinoma (PRCC) occurs in sporadic and hereditary forms.¹ There are two histological subtypes; type 1 with chromophil basophilic histology, and type 2 with chromophil eosinophilic histology.² Sporadic PRCC is usually solitary and unilateral, and ~75% of tumours are associated with trisomy 7. Somatic trisomy 16, 17, loss of the Y chromosome and t(x;1)(p11.2;q21.2) have also been described. Hereditary PRCC is characterised by multiple, bilateral tumours, type 1 histology and autosomal dominant transmission with reduced penetrance.¹ Somatic and germline mutations in the met proto-oncogene (MET; 7q31) have been described in PRCC, with the majority of described germline mutations occurring in the presence of type 1 histology.³⁵

Case report: Following detection of a unilateral renal mass, KN had a left nephrectomy at 52 years. Histopathology confirmed a large tumour (55mm in diameter) in the superior pole, multiple smaller subcapsular tumours, and PRCC with predominantly papillary architecture and areas of clear cell pattern. He represented at 56 years with a mass involving his right kidney. More than 20 separate larger cortical tumours (>5mm diameter) and numerous small cortical and subcapsular tumours (<5mm diameter) were evident in the right nephrectomy specimen. Histopathology confirmed PRCC with type 1 histology and areas of clear cell pattern. There was no known family history of renal cancer.

Genetic studies: Bi-directional genomic DNA sequencing detected a single germline nucleotide substitution (HGVS NM_000245.2(MET):c.3658G>A and NP_000236.2(MET):p.Val1220Ile) in MET. Evidence in the published literature, pedigree data and in vitro functional studies are consistent with this sequence variant being pathogenic.⁴ ⁶ ⁷ The mutation has been inherited by one of KN’s three sons, who has a normal renal ultrasound.

Conclusion: Genetic testing in MET is important in individuals with PRCC, particularly those with multiple and/or bilateral tumours, type 1 histology, young age at diagnosis and/or a family history of PRCC.

NOVEL HERITABLE MUTATION OF THE TRANSCRIPTION FACTOR RUNX1 AS A CAUSE OF AUTOSOMAL DOMINANT FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO ACUTE MYELOID LEUKAEMIA (FPD/AML)

Nigel Patton [1], Graeme Suthers [2], Meryl Altree [2], Jacqueline Carroll [1], Carolyn Butcher [3], Catherine Carmichael [4], Ella Wilkins [4], Richard D'Andrea [3], Hamish Scott [4]


Aim
To identify the causative heritable mutation in a family with autosomal dominant familial platelet disorder with predisposition to acute myeloid leukaemia (FPD/AML).

Method
Confirmation of family pedigree, enrolment into ethics committee approved Australian Familial Haematological Cancer Study, procurement of genomic DNA from pedigree members, and genetic analysis by sequencing of RUNX1 and CEBPA genes.

Results
The proband presented at age 50 with thrombocytopenia initially diagnosed as ITP; bone marrow examination (including cytogenetics) was normal. Three years later she developed progressive severe thrombocytopenia and mild anemia/neutropaenia. Marrow examination revealed monosomy 7 in 12/20 metaphases, and a diagnosis of myelodysplastic syndrome (MDS) was made. Six months later (Feb 2007), she progressed to AML. The proband's mother had had mild thrombocytopenia with subsequent MDS (aged 70) and died of AML two years later. The proband's only sibling and nephew have mild thrombocytopenia without features of MDS. The proband entered cytogenetic remission following AML induction therapy and is awaiting unrelated donor allogeneic stem cell transplant. Sequencing of PCR products from exons of the RUNX1 gene identified a heterozygous mutation in the proband's constitutional DNA: c.958C>T in exon 7 causing a nonsense mutation, p.Arg293X. The sibling has the same mutation, and other studies in relatives are underway.

Conclusion
This study has identified a novel RUNX1 mutation responsible for FPD/AML in this family. Clinicians should be aware of this rare inherited disorder and the availability of genetic testing. People with mutations may be asymptomatic, and genetic testing in such families is essential before considering bone marrow transplantation from a living related donor.
TARGETED DISRUPTION OF BRCA1 IN RESTRICTED COMPARTMENTS OF THE MOUSE MAMMARY EPITHELIA: DOES A LACK OF PHENOTYPE HAVE IMPLICATIONS FOR THE ORIGIN OF BRCA1-TUMOURS?

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The basal-like phenotype of breast tumours observed in BRCA1 mutation carriers has prompted speculation about their cellular origin. One current hypothesis is that BRCA1 plays a specialised and important role either in the basal-cell compartment or the stem cell compartment of the mammary epithelia and that disruption of this function may contribute to tumourigenesis in these cells. To address this hypothesis, we used the Cre-loxP system to target Brca1 disruption to K14 and K6a expressing cells, which include basal cells and the putative progenitor cells of the mammary gland respectively. We then examined the effect of this on mammary gland development and tumourigenesis. Unlike MMTV and WAP driven conditional knockout models of Brca1 loss, disruption in K14 and K6a expressing cells and their progeny, did not result in any observable changes to pubertal development. Furthermore, the K14 model did not exhibit lactational defects or preneoplastic or neoplastic changes within 15 months. Our results suggest that the developmental defects previously observed in conditional knockouts of Brca1 are unlikely to be due to the consequences of Brca1 disruption in K14-positive basal cells or the putative K6a-positive putative progenitor or stem cells. The lack of phenotype observed in this model suggests that Brca1 does not play an important role in the K14 or K6a positive compartment during mammary gland development. Furthermore, our results may support an alternative hypothesis of Brca1 induced trans-differentiation of K14 and K6a negative cells in mammary tumourigenesis.
#28 BRCA1 AND BRCA2 TESTING IN MALE BREAST CANCER PROBANDS: THE ADELAIDE EXPERIENCE

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1South Australian Familial Cancer Service, Women's and Children's Hospital, North Adelaide, Australia; 2University Department of Paediatrics, University of Adelaide, Australia; 3Kathleen Cuningham Foundation Consortium for research into Familial Breast Cancer, Australia; 4Genetic Pathology Laboratory, Flinders Medical Centre, Bedford Park, Australia; 5Molecular Pathology Division, Institute of Medical and Veterinary Science, Adelaide, Australia.

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AIM AND METHOD: To review the outcome of BRCA1/2 testing in male probands who experienced breast cancer and were the first individual in their family to be tested.

RESULTS:

Probands: Twenty male probands with unilateral breast cancer were tested under “Adelaide Criteria 5.1” (male breast cancer at any age), including 2 tested concurrently with a female relative who had experienced breast cancer. Proband breast cancers were diagnosed at average 58 years (range 35-79 years). Fifteen had experienced breast cancer only and 5 had experienced breast and another cancer (1 CRC, 1 melanoma, 1 renal adenocarcinoma, 1 Wilms and 1 melanoma +prostate).

Family History: One proband was adopted. All 19 with an available FmHx reported cancers in family members (average 5/family, range 1-26). Regarding breast cancer; 7 (37%) had no FmHx of breast cancer, 5 (26%) had 1 affected female relative and 4 (21%) had 2 affected female relatives. One proband each had 4 and 6 female relatives with breast cancer. One proband had 8 relatives with breast cancer - 6 females (1 with bilateral disease) and 2 males. Five prostate and 6 gastric cancers were reported, but no ovarian or pancreatic cancer. Manchester BRCA2 scores were ≥10 in only 5/19 (26%).

BRCA Analysis: Considering all males tested: a BRCA2 mutation was detected in 7/20 (35%) and an equivocal BRCA2 variant in 1 (5%). Considering subgroups: a BRCA2 mutation was identified in 2 of 12 (17%) with a FmHx of breast cancer in 0 or 1 female relative, 3/16 (18%) with a FmHx of a BRCA2 cancer in only 0-2 relatives and 2/14 (14%) with a Manchester BRCA2 score <10.

CONCLUSION: Our small data set suggests BRCA2 mutation testing may be indicated for males who experience breast cancer, even when the family history of BRCA2 related cancers is unremarkable.

Table: additional details about BRCA2 mutation carriers

<table>
<thead>
<tr>
<th>mutation type(s)</th>
<th>Proband Cancer (age)</th>
<th>FmHx BRCA2 cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Man score</td>
<td>breast</td>
</tr>
<tr>
<td>NS</td>
<td>Breast + CRC (54 + 69 yr)</td>
<td>34</td>
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<tr>
<td>EV + NS (cis)</td>
<td>Breast (59 yr) (adopted)</td>
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<td>NS</td>
<td>Breast (66 yr)</td>
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</tr>
<tr>
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<td>Breast (68 yr)</td>
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<td>del exon 19</td>
<td>Breast (56 yr)</td>
<td>25</td>
</tr>
<tr>
<td>FS</td>
<td>Breast (66 yr)</td>
<td>13</td>
</tr>
<tr>
<td>EV + EV (phase)</td>
<td>Breast + Wilms (43 + 7 yr)</td>
<td>9</td>
</tr>
</tbody>
</table>

* EV=equivocal variant; NS=nonsense; MS=missense; FS=frameshift, Man score = Manchester BRCA2 score

“Familial Cancer 2007: Research and Practice”
#29
THE ROLE OF THE ATM GENE IN FAMILIAL BREAST CANCER

Sue Healey\(^1\), Anne Hartmann\(^2\), Barbara Herte\(^2\), Stephanie Distler\(^2\), Xiao Qing Chen\(^1\), kConFab, Saundra Buys\(^3\), Irene Andrulis\(^3\), Mary Daly\(^3\), John Hopper\(^3\), Esther John\(^3\), Mary Beth Terry\(^3\), Peter Oefner\(^2\), Georgia Chenevix-Trench\(^1\)

\(^1\)Queensland Institute of Medical Research; \(^2\)Institute of Functional Genomics, Regensburg University; \(^3\)Breast Cancer Family Register

The role of the \textit{ATM} gene in breast cancer predisposition is controversial. Studies of carriers of \textit{ATM} mutations have indicated that females have on average a 2 - 7 fold increased risk of breast cancer, and overall \textit{AT}-causing mutations have a relative risk of 2.37 for breast cancer [Renwick, 2006] but, apart from \textit{ATM} 7271T>G where the confidence intervals are still very wide, the penetrance of specific mutations is not known.

In September 2005, NIH funded a major collaborative project (PI: Georgia Chenevix-Trench) involving eleven sites and several independent consultants, to investigate the role of the \textit{ATM} gene in familial breast cancer. Our main aim is to estimate the frequency and penetrance of \textit{ATM} mutations in a large number of putative hereditary breast cancer families who have been recruited by kConFab and the Breast Cancer Family Register (BCFR) in North America and Australia.

Work on four main areas of interest is now in progress:

1. Mutation screening (sequence analysis and MLPA) of the \textit{ATM} gene in the youngest affected female from 950 breast cancer families, all of whom have a ‘Manchester score’ for \textit{BRCA2} of more than 5. Sequencing of the first 380 samples has identified 9 \textit{AT}-causing mutations, as well as 46 unclassified variants.

2. Identification of “putative” \textit{ATM} mutations among the variants found using a variety of methods including the determination of allele frequencies in 380 population control samples from the Australian Cancer Study (matched to the kConFab cases), evaluation of loss of heterozygosity in breast tumours, the Protein Truncating Test (for putative splice site mutations) and expression profiling of lymphoblastoid cell lines after ionizing radiation.

3. Genotyping the affected and unaffected family members of those individuals found to carry “putative” \textit{ATM} mutations, and performing modified segregation analyses in order to estimate the penetrance.

4. Screening for the “putative” \textit{ATM} mutations in an additional 4,700 breast cancer cases and 2,200 controls (from BCFR and the Australian Red Cross Blood Bank) using iPLEX Mass Array.
RAPID DETECTION OF CARRIERS WITH BRCA1/2 ASHKENAZI MUTATIONS USING HIGH RESOLUTION MELTING; IMPLICATIONS FOR SCREENING BREAST CANCER PREDISPOSITION.

Alexander Dobrovic¹, Elena Takano¹, Victoria Girgis¹, Serge Kovalenko¹, Gillian Mitchell² and Stephen Fox¹. ¹Department of Pathology, ²Familial Cancer Centre, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia.

BRCA1 and BRCA2 are the two most frequently mutated genes underlying inherited breast/ovarian cancer cases. As both are large multi-exon genes, with inactivating mutations occurring across the entire coding region, the consequent costs of BRCA1/2 mutation screening has limited it to those who are most likely to have a mutation based on family history. Consequently, a significant proportion of women carrying germline mutations are missing out on the opportunity to have mutation screening.

High resolution melting (HRM) is a new methodology for mutation scanning in which the mutation scanning is carried out in the same tube that the sequence is amplified in. The PCR reactions and high resolution melting can all be performed in a single run of less than 2 hours. Up to 384 scans can be done in the same time resulting in extremely rapid screening. We are developing HRM assays encompassing the coding sequences of the BRCA1 and BRCA2 genes. The primer design is done to avoid complex melting domains in single amplicons and making sure that the primers do not overlay SNPs that may lead to mutant alleles not being amplified. The PCRs are being designed so that they all operate under the same cycling conditions.

HRM is not only the least expensive screening method but also one with 100% sensitivity as each heterozygous mutation creates a readily detected heteroduplex. As evidence for this, we have tested multiple exons including the 3 bearing the Ashkenazi mutations that all run simultaneously. 100% sensitivity of mutation detection has so far been achieved. The results must be sequence verified to distinguish mutations and neutral variants and SNPs but this can be performed in a timely manner due to the limited sequencing required. HRM can also be used as an inexpensive methodology for tracking mutations across pedigrees. HRM should become the method of choice for rapid and inexpensive screening of the BRCA1 and BRCA2 genes.
HIGH RESOLUTION MELTING FOR MUTATION SCANNING OF TP53 EXONS 5-8

Michael Krypuy1, Ahmed Ashour Ahmed2, Dariush Etemadmoghadam3,4, Australian Ovarian Cancer Study Group, Sarah J Hyland5, James D Brenton2,5, Stephen Fox1,6, David D Bowtell3,4, Alexander Dobrovic1,6§

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2 Functional Genomics of Drug Resistance Laboratory, Cambridge Research Institute, Cambridge CB2 0RE, UK.
3 Ian Potter Centre for Genomics and Predictive Medicine, Peter MacCallum Cancer Centre, Locked Bag 1, A'Beckett St, Melbourne, Victoria 8006, Australia.
4 Department of Biochemistry and Molecular Biology, University of Melbourne, Parkville, Victoria, 3010, Australia.
5 Hutchison/MRC Research Centre, University of Cambridge, Cambridge
6 Department of Pathology, University of Melbourne, Parkville, Victoria, 3010, Australia.

The TP53 gene is commonly inactivated by mutations in the DNA-binding domain (exons 5 to 8) in a wide range of cancers. As p53 mutation often influences response to therapy, effective and rapid methods to scan for mutations in TP53 are likely to be of clinical value in breast and ovarian cancers. We therefore evaluated the use of high resolution melting (HRM) as a mutation scanning tool for TP53 in tumour samples. We designed PCR amplicons for HRM mutation scanning of TP53 exons 5 to 8 and tested them with cell lines with known mutations. We tested the sensitivity of each PCR amplicon with dilutions of mutant cell line DNA in normal wild-type DNA. We then performed a blinded assessment on 20 ovarian tumour DNA samples that had previously sequenced for mutations in TP53, to assess the sensitivity and specificity of the technique. Cell line sensitivity testing showed that 5% of mutant DNA was detectable for each amplicon using HRM. Aberrant HRM curves indicative of p53 mutations were observed for each of the 20 samples in the ovarian tumour DNA panel. The method was then further validated using a panel of previously untyped breast cancer DNAs. Comparison of the HRM results with the DNA sequencing results revealed that each mutation was detected by HRM in the correct exon. HRM is an ideal technique for simple and rapid scanning for TP53 mutations that would also be particularly applicable to Li-Fraumeni p53 germline mutation carriers.
**CHD5 MUTATION SCREENING IN BREAST AND OVARIAN TUMOURS USING HIGH RESOLUTION MELT ANALYSIS.**

Kylie L Gorringe, David YH Choong, Ian G Campbell

VBCRC Cancer Genetics Laboratory, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia.

**Introduction:** Chromodomain, helicase, DNA binding domain 5 (CHD5) is a putative tumour suppressor gene located on chromosome 1p, a region with high levels of LOH in ovarian cancer. A recent sequencing study identified heterozygous mutations in breast cancer (Sjöblom et al., 2006). Functional evidence from mouse models supports a potential tumour suppressive role for the CHD5 protein (Bagchi et al., 2007).

**Methods:** High Resolution Melt (HRM) analysis is a relatively new technique for mutation detection that exploits differences in double stranded DNA dissociation temperature (melting profile) caused by sequence changes. Measurement of the melting profile uses an intercalating dye that is fluorescent when incorporated into double stranded DNA but is released upon melting resulting in a measured loss of fluorescence. Subtle shifts in the temperature at which the dye is released indicate sequence alterations. Using the Roche LightCycler, we have employed this method to mutation screen CHD5 in 100 ovarian and 30 breast tumours. Each exon and splice junction was amplified in 150-200 bp fragments from 10 ng of whole-genome amplified tumour DNA.

**Results:** While the analysis of this 41 exon gene is still incomplete, we have readily been able to detect many different polymorphisms and a single somatic missense mutation. We find HRM to be a very sensitive method for the detection of known SNPs and also for mutation screening, however, the CHD5 gene does not appear to be a major tumour suppressor gene in breast or ovarian cancer.


#33

**DMBT1 AS A POTENTIAL GENETIC MODIFIER OF BREAST CANCER RISK.**

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DMBT1 (deleted in malignant brain tumours) has been identified as a candidate modifier gene for breast cancer through a genetic mapping study in mice heterozygous for p53 (Trp53+/-) (Am J Path, 170:2030 –2041, 2007). Low levels of DMBT1 expression were associated with increased risk of breast cancer in both mice and women. We have now investigated several SNPs in potential regulatory regions of DMBT1 for association with breast cancer risk in population studies and for functional effects on gene expression in luciferase reporter assays.

Population investigated were BRCA1 or BRCA2 mutation carriers accessed via the Consortium for Investigators of Modifiers of BRCA1/2 (CIMBA)(including kConFab samples), kConFab familial breast cancer cases without BRCA1/2 pathogenic mutations, cancer-free controls recruited into the Australian Ovarian Cancer Study (AOCS), population-based breast cancer cases and controls recruited into the Australian Breast Cancer Family Study (ABCFS).

Sequencing and cloning of the 5′ promoter of DMBT1 has found only 2 common haplotypes, consisting of 17 SNPs over 2.2kb upstream. SNP rs2981739, a tag SNP for the haplotypes which alters a progesterone response element (PRE) at −1224 bp, was examined. No significant association was found with breast cancer risk in any populations genotyped. Consistent with this, luciferase reporter assays on the 2.2 kb region in HEK cells did not find any major difference in basal promoter activity between the haplotypes.

Sequencing and cloning of the 3′ untranslated region (UTR) of DMBT1 has found 3 common and 1 rare haplotypes, (frequencies 0.61, 0.23, 0.14 and 0.03), defined by 3 SNPs in the 337 bp region. SNP rs8441, which delineates only 1 of the 4 haplotypes, was associated with increased breast cancer risk in BRCA2 carriers (RR homozygote 1.7, 1.0-3.0, ptrend=0.04). A similar non-significant trend was observed for non-carriers. Risk of breast cancer in BRCA1 carriers was inconsistent (ptrend=0.1), with decreased risk for heterozygotes (RR 0.8, 0.7-0.9) but no significant risk for homozygotes (RR 1.0, 0.7-1.4). Preliminary functional testing of the effect of the 3′UTR haplotypes in luciferase reporter assays in HEK cells indicates there is no major difference on basal expression between the haplotypes.

DMBT1 remains a candidate modifier gene of breast cancer. Studies on additional polymorphisms and functional testing of induced expression in breast epithelial cells are required to understand the possible contribution of DMBT1 to breast cancer risk. Supported by NHMRC 179842 Howard Florey Fellowship and NHMRC 366787 R.D. Wright Career Development Award.
A SIGNIFICANT INCIDENCE OF CANCERS OTHER THAN THOSE OF THE BREAST AND OVARY IN KCONFAB FAMILIES

Nicci Wayte¹,², David Duffy¹ and Georgia Chenevix-Trench¹

¹Queensland Institute of Medical Research, Brisbane
²The School of Molecular and Microbial Bioscience, The University of Queensland

kConFab ascertains families with multiple cases of breast and ovarian cancer, and about 34% of these families have mutations in BRCA1 or BRCA2. Families with hereditary breast and ovarian cancers are also at increased risk to other cancers, including cancers of the pancreas and colon for BRCA1 mutation carriers [1-3] and cancers of the pancreas, prostate, gall-bladder, bile duct, stomach and malignant melanoma for BRCA2 mutation carriers [4-9].

We hypothesise that some non-BRCA1/2 kConFab families, particularly those with many cases of cancer of diverse types, may be caused by rare mutations in other, yet to be identified, cancer susceptibility genes that give rise to a wide spectrum of cancers. We further hypothesize that such ‘general cancer susceptibility genes’ are likely to be tumour suppressor genes in which the wild-type allele undergoes allelic loss and thus may also contribute to sporadic cancer if both alleles are inactivated somatically. Here we present our statistical analysis of non-breast and ovarian cancers in non-BRCA1/2 kConFab families and the adaptation of this method to identify families with a significant excess of multiple cancer types. We found a significant incidence of prostate, colorectal and brain cancers across a subset of non-BRCA1/2 kConFab families. We also identified a number of individual non-BRCA1/2 families in kConFab with a significantly increased incidence of multiple different cancer types, in addition to breast and/or ovarian cancers. In particular, one kConFab family identified has a significant excess of breast, melanoma and colorectal cancers in addition to six other cancer types occurring in 1-2 cases. 38 blood samples and 14 tumour samples are potentially available from this family, making it a suitable candidate for both linkage and concurrent copy number and loss of heterozygosity (LOH) analyses. We plan to use these approaches in conjunction with pathological data to identify novel tumour suppressor genes in kConFab families. To this end, we will present results from preliminary LOH and copy number analysis of archival tumours.

USE OF EXPRESSION DATA FROM IRRADIATED LYMPHOBLASTOID CELL LINES AND CGEMS BREAST CANCER GENOME-WIDE ASSOCIATION SCAN DATA TO IDENTIFY GENES THAT MAY MODIFY RISK IN BRCA1 AND BRCA2 CARRIERS.

Logan C. Walker¹, Nicola Waddell¹, kConFab Investigators², Sean Grimmond³, Amanda B. Spurdle¹

¹Queensland Institute of Medical Research, Australia ²Peter MacCallum Cancer Centre, Melbourne, Australia ³Institute for Molecular Biosciences, University of Queensland, Australia

Approximately 5-10% of breast cancer cases occur in high risk families, and are attributable to inherited mutations of highly penetrant breast cancer susceptibility genes, including BRCA1 and BRCA2. However, individualised risk prediction in mutation carrying families is complicated by the observation that phenotypic expression of inherited disease can differ between families carrying the same pathogenic mutation. Although this observation may be explained in part by environmental variation, studies have also presented epidemiological evidence that other genes harbouring polymorphisms or mutations may modify risks in BRCA1 and BRCA2 carriers. The best candidate ‘modifier’ gene to date is possibly RAD51, which has been shown by independent studies to increase breast cancer risk in Ashkenazi Jewish BRCA2 mutation carriers.

As part of a separate study, lymphoblastoid cell lines from BRCA1 or BRCA2 mutation carriers and mutation-negative controls had been subjected to ionizing irradiation, and the cellular response measured using expression microarray analysis. We explored the value of analysing gene expression differences in this dataset to prioritise candidate modifier genes for polymorphism association studies. Genes that discriminated between BRCA1 or BRCA2 mutation carriers and mutation-negative controls were identified and filtered based on cellular function. These refined gene lists were then aligned against data derived through the publicly available genome-wide association study of breast cancer, Cancer Genetics Markers of Susceptibility (CGEMS). Results of this analysis will be presented along with a discussion of this approach to identify candidate modifiers of BRCA1 and BRCA2.
REGULATION OF SPLICING AND ALTERNATIVE SPLICING OF THE BRCA GENES

Christopher A Pettigrew1, Chanel E Smart1, Nicola Wayte1,2, Therese Lundstrom1, Ania Wronski1, Paul K Lovelock1,2, Juliet D French1, Jodi M Saunus1, Joseph A Rothnagel1, Amanda B Spurdle2, Melissa A Brown*1

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2 Cancer and Cell Biology Unit, Queensland Institute of Medical Research, Herston, Queensland, Australia.

Splicing is an integral component of post-transcriptional processing of pre-mRNA transcripts. Dis-regulation of splicing may lead to the production of unstable transcripts or aberrant protein products and is strongly associated with multiple diseases, including cancer. Splicing is controlled by sequence elements at the intron-exon boundaries of genes (consensus splice sites) as well as regulatory elements within exons (ESEs). The breast cancer susceptibility genes (BRCA) are tumour suppressor genes that have a wide range of functions including DNA repair, transcriptional activation and chromatin remodelling. Disruption of either BRCA1 or BRCA2, by mechanisms including aberrant splicing has been associated with breast cancer predisposition. Splicing abnormalities include mutation of consensus splice sites and ESEs and imbalances in the expression of alternative splice variants. The precise contribution of splicing defects in BRCA1 and BRCA2 to human disease however is currently unknown, largely as a result in limited knowledge of all of the functional ESEs that exist in these genes and the exact nature and function of the splice variants they produce. To address these issues, we have developed a novel approach for prioritizing predicted ESEs in BRCA1 and BRCA2, based on evolutionary conservatism and colocalization with reported sequence changes. Results on BRCA1 have been presented at this meeting previously. Results with BRCA2 will be presented this year. To begin to identify and characterize the splicing products of BRCA genes, an RT-PCR screen of mouse mammary tissue has been performed. This presentation will include data on the sequence, expression and functional activities of several novel Brca1 splice variants.
ALTERNATE MECHANISMS FOR BRCA1 SILENCING IN YOUNG WOMEN WITH BREAST CANCER

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1Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, 2Molecular Pathology Research, Peter MacCallum Cancer Centre, 3The Australian Breast Cancer Family Study, 4School of Molecular and Microbial Sciences, The University of Queensland, 5Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne 6Department of Pathology, The University of Melbourne

Hereditary breast cancers constitute approximately 10-15% of all breast cancers (Easton et al., 1995) and only a small proportion diagnosed in women younger than 40 years can be explained by germline mutations in BRCA1. A population-based study of Australian women has shown that BRCA1 germline mutations account for 3.8% (95% CI 0.3-12.6%) of all breast cancers diagnosed under the age of 40 years (Southey et al., 1999) and for only 15% of those women with a strong family history (Dite et al., 1999).

Breast cancers arising in women with germline BRCA1 mutations have distinctive morphological and molecular features. In our study of early-onset breast cancer, 95% of BRCA1-mutation associated tumours have the “BRCA1 mutation-associated” morphology (Armes et al., 1999; unpublished data). However, there is a substantial proportion (approximately 19%) of all early-onset breast cancer that have BRCA1-like morphology but have no identifiable BRCA1 germline mutation. We investigated possible alternate mechanisms of BRCA1 inactivation in these early-onset BRCA1-like breast cancer cases.

We identified women from The Australian Breast Cancer Family Registry (Breast CFR) who were diagnosed with

1) breast cancer before the age of 40 years and had a strong family history (n=66) and/or
2) had tumour morphology consistent with having a BRCA1 mutation (n=76).

These women had undergone extensive prior mutation screening for BRCA1 mutations via a variety of methods (Dite et al., 2003).

We screened the germline DNA for large genomic alterations in BRCA1 using multiplex ligation-dependent probe amplification (MLPA) and have identified a variety of large genomic alterations involving the coding regions of BRCA1 and/or the promoter regions that represent approximately 25% of all BRCA1 mutations identified in these women.

We have also examined the frequency of BRCA1 promoter methylation in our early-onset breast cancer cases with a BRCA1-like morphology against that of age-matched affected women that did not have a BRCA1-like tumour morphology using Methylation-specific High Resolution Melting (MS-HRM) analysis. As expected many of the DNA from tumour specimens were of poor quality so we designed a specific assay that would still enable the determination of BRCA1 promoter methylation. A small proportion of the BRCA1-like tumours showed BRCA1 methylation, indicating that methylation was not the principal cause of BRCA1-like morphology in mutation-negative tumours. Further investigations (such as mutation scanning in the regulatory regions of BRCA1) are underway to determine the origin of BRCA1-like morphology in the remaining tumours.
A genetic disease is said to exhibit a parent-of-origin effect (POE) if it is largely controlled by a single autosomal locus; it is dominantly-inherited; and its severity in the offspring of a female carrier is on average different to that of a male carrier. Fragile-X and Huntington’s disease each exhibit a POE. A POE can be caused by a number of non-Mendelian mechanisms, including genomic imprinting and repeat-sequence expansion. Many diseases which exhibit a POE also display genetic anticipation (and vice-versa) so testing for one can often be done in place of testing for the other. Testing for a POE instead of anticipation is attractive because many tests for anticipation are biased by uncontrollable factors and because the notion of genetic anticipation is only nebulously defined.

We review some common statistical tests for parent-of-origin effects and genetic anticipation and present a new POE test. Our test is unaffected by cohort effects, differential age-censoring between generations, population stratification or simple disease sex-differences. It allows rigorous conditioning for ascertainment and can be readily implemented using the program MENDEL.
OVARIAN EPITHELIAL CELL GENE PROFILES IN VIVO, AND DYSREGULATION IN OVARIAN ADENOCARCINOMA.

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The most consistent risk factors for sporadic ovarian cancer are related to reproduction, with a higher number of life-time ovulations increasing risk and pregnancy conferring some protection. Ovulation involves the processes of proliferation, apoptosis, wound-repair and inflammation. Aberrations in each of these features have been implicated in the pathway to malignancy in a variety of human organ systems and we reasoned that dysregulation in these normal processes may contribute to neoplastic transformation of ovarian epithelial cells. Previous work in our laboratory has identified genes expressed and regulated in normal ovarian epithelial cells during the estrous cycle in mice. We compared these gene profiles with genes found to be expressed in human epithelial ovarian cancer in published microarray studies. Overall, we identified 166 genes that were regulated during the estrous cycle and consistently dysregulated in ovarian cancer. Analysis of the predicted ontologies of genes which overlapped between ovarian cancer and normal mouse OSE revealed over-representation of a number of processes, including those involved in the cell cycle (P<0.003; Fisher’s Exact Test, GOstat), transport (P<0.006), assembly and arrangement of cell structures (P<0.02), and metabolism (P<0.04). The pathways with the highest number of genes from the overlapping set included the MAP kinase signalling pathway, cell cycle, cell and focal adhesion, regulation of the actin cytoskeleton and leukocyte trans-endothelial migration (KEGG pathway database).

We are currently validating a subset of these genes in human ovarian adenocarcinoma, low malignant potential and benign disease as well as in normal ovarian tissue, by immunohistochemistry. The candidates under investigation include known and novel ovarian cancer-related genes: epithelial-cell adhesion molecule (EpCAM), mitogen activated protein kinase 1 (MAPK1), lipocalin-2 (LCN2), enhancer of zeste homolog 2 (EZH2), SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4 (SMARCA4), p21 activated kinase 2 (PAK2), survivin and NUAK2 (NUAK family, SNF1-like kinase, 2), a gene with a high probability of having a ‘driver’ mutation in both breast and ovarian carcinomas.

Preliminary data indicates that EpCAM, MAPK1, EZH2, survivin and SMARCA4 are all expressed at moderate to high levels in the majority of ovarian adenocarcinomas, whereas LCN2 expression was generally low.

This approach, defining overlapping gene expression profiles between normal and malignant ovarian epithelial cells has lead to the identification of subsets of genes and functional pathways that are involved in normal OSE functions that may contribute to the development of human EOC and represent promising candidates for ovarian cancer detection, diagnosis and therapy.
THE DUAL SPECIFICITY PHOSPHATASE DUSP26 IS A CANDIDATE TUMOUR SUPPRESSOR IN OVARIAN CANCER.

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Dysregulation of mitogen-activated protein kinase (MAPK) signalling pathways is commonly implicated in the pathogenesis of cancer, including epithelial ovarian cancer which is the most lethal gynaecological malignancy. Dual specificity phosphatases (DUSP) play a key role in MAPK regulation via inactivation of specific MAPKs by selective dual dephosphorylation of phospho-tyrosine and phospho-threonine residues within the kinase activation loop. DUSPs frequently exhibit reduced expression in cancer and many are implicated as tumour suppressor genes. Using expression profiling of primary ovarian cancers, we identified a novel member of the DUSP family, DUSP26, as having down-regulated expression in ovarian cancer compared to normal ovaries. DUSP26 is located at 8p12, a region associated with allelic imbalance in ovarian cancer and postulated to encode at least one unidentified tumour suppressor gene. Quantitative real-time RT-PCR analysis in cell lines and primary tumours confirmed that DUSP26 is down-regulated in all histological subtypes of epithelial ovarian cancer. The aim of this study was to determine the effect of DUSP26 on MAPK pathways and epithelial cell growth and survival.

Recombinant DUSP26 was able to dephosphorylate the phosphatase substrate pNPP in vitro, confirming its activity as a phosphatase. To identify if DUSP26 has activity against key MAPK substrates, we determined relative phosphorylation levels of ERK, JNK and p38 following transfection of COS7 cells with DUSP26, and following direct incubation of recombinant ERK, JNK and p38 MAPK with recombinant DUSP26, using Western blotting and phospho-specific MAPK antibodies. DUSP26 did not consistently dephosphorylate ERK, JNK or p38 in any of the assay systems. Overexpression of DUSP26 but not of a catalytically inactive mutant of DUSP26 reduced the colony forming ability of epithelial cells. Conversely, siRNAs directed against DUSP26 enhanced both the colony forming ability and proliferation rate of immortalised human ovarian surface epithelial cells.

Together, these results show that DUSP26 has activity consistent with a tumour suppressor gene, however, unlike most DUSPs characterised to date, MAPKs are likely not the primary substrate of DUSP26. We are currently investigating other potential kinase substrates of DUSP26 using a substrate trapping approach.
OVARIAN PHENOTYPE OF HOMOZYGOUS ATM 7271T>G KNOCK-IN MICE.

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Ataxia telangiectasia (A-T) is a human autosomal recessive disorder in which patients show ataxia, increased sensitivity to radiation and increased susceptibility to lymphoid and other cancers. Mutation or deletion of the “ataxia-telangiectasia-mutated” (ATM) gene responsible for A-T also results in severe meiotic disruption in humans, leading to infertility. Deletion of the Atm gene in mice blocks gametogenesis and leads to a lack of oocytes and follicular structures in homozygous Atm−/− adult female animals. We have generated Atm 7271 T>G homozygous mice which have a knock-in point mutation equivalent to a T to G transition at position 7271 in human ATM. This results in a milder A-T phenotype than the full knock-out. Here we report aspects of the ovarian phenotype in the Atm 7271 T>G homozygous mice.

The adult ovaries of these transgenic mice were small and fetal-like, with embryological characteristics including cords of cells without apparent follicular structures. Ovaries of three two-month old Atm 7271 T>G mice were characterised using immunohistochemistry for GDF-9 (goat polyclonal IgG, Santa Cruz Biotechnology sc-12244), a germ cell and oocyte marker and inhibin-alpha (Serotec anti-human inhibin-alpha, Clone R1), a granulosa cell marker. Three-month old control ovaries showed GDF-9 immunoreactivity only in oocytes, and inhibin-alpha immunoreactivity in the granulosa cells of primordial to preovulatory follicles. In Atm 7271 T>G ovaries, positive GDF-9 staining was noted in a small number of discreet ovarian cells and in one case, in adjacent connective tissue. Inhibin-alpha immunoreactivity was observed in clusters of cells within the Atm 7271 T>G ovaries. In one structure, positive GDF-9 staining, but no inhibin-alpha staining was observed.

We conclude that the Atm 7271 T>G mutation causes a less severe ovarian phenotype than the full Atm knockout, resulting in a delay in degeneration of oocytes in the adult ovary and possibly a delay in ovarian development.
VALIDATION OF THE LAMBDA MODEL FOR ASHKENAZI JEWISH WOMEN

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Approximately 1 in 40 Ashkenazi Jewish (AJ) women carry a deleterious mutation in one of the cancer-related genes BRCA1 or BRCA2. LAMBDA is a model which estimates an AJ woman’s probability of carrying such a mutation from her family history of breast and ovarian cancer. LAMBDA is easy to use compared with other genotype-prediction models so it is likely to be increasingly popular in clinical practice. Therefore it is important that its validity be properly assessed.

We studied the performance of LAMBDA, developed using Australian and British data, on 1,286 North American AJ women. We found it performed at least as well as, and on some grounds better than, the acclaimed genotype-prediction model BRCAPRO. Therefore LAMBDA can be regarded as a simple but effective clinical tool to triage AJ women for genetic testing.
HOW ACCURATE ARE WE? AN ASSESSMENT OF THE ADELAIDE CRITERIA FOR BRCA1 AND BRCA2 MUTATION SEARCHING.

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BACKGROUND: The American Society of Clinical Oncology recommends BRCA1/2 mutation searching is offered to cancer affected individuals when the probability of identifying a mutation is 10% or greater.¹ Using published clinical experiences and recommendations, and aiming to optimise BRCA1/2 mutation detection, the South Australian Familial Cancer Service (SAFCS) generated clinical criteria for triaging BRCA1/2 mutation searching in cancer affected probands. These Adelaide Criteria divide probands into 8 groups (with 19 subgroups).

AIM: To review the SAFCS’s BRCA1/2 mutation detection rate and to identify which (sub)groups of the Adelaide Criteria fall below the 10% mutation probability threshold.


RESULTS: BRCA searching: BRCA mutation searching was completed in 729 probands, 39 who had BRCA1 testing only, 27 BRCA2 testing only and 663 testing at both loci. Overall 131 (17.9%) probands had a BRCA1 (N=72) or BRCA2 (N=60) mutation identified, including one woman with a mutation of both loci. Mutation probability threshold: Where >10 probands were tested, only one subgroup fell below the 10% mutation probability threshold – subgroup 7.1 (20/367; 5.4% had a mutation detected). Woman in this subgroup have experienced breast or ovarian cancer and meet the National Breast Cancer Council definition for "high genetic risk", but do not fit any of the other Adelaide Criteria subgroups - they often have a family history restricted to 3 breast cancers in closely related older women, or 2 breast cancers where 1 woman was <50 at cancer diagnosis. Presymptomatic tests generated: The 131 women with an identified mutation have generated 1369 formal risk notifications to relatives over the age of 18 (average 10.4 per family). A total of 441 presymptomatic tests have been generated (average 3.3 per family). There is a trend for more formal risk notifications and more presymptomatic testing to occur in families with a greater number of women affected by breast cancer. Subgroup 7.1: The 20 women from subgroup 7.1 with identified mutations represent 15.1% of mutations detected, 15.2% of risk notifications made and 16% of presymptomatic tests generated. However, 367 women were tested to identify these 20 women, representing 50.3% of the total number of BRCA1/2 mutation searching tests performed.

CONCLUSION: Overall the Adelaide Criteria are performing well (mutation detection 17.9%) and all subgroups except 7.1 exceed the 10% mutation probability threshold. Subgroup 7.1 clearly limits the usefulness of the Adelaide Criteria, and it can be argued that women in the subgroup should not be tested (mutation detection 5.4%). However, eliminating this subgroup would result in a significant number of mutations being missed (15% of our cohort). An alternative method for triaging BRCA1/2 mutation searching is needed, particularly for the 7.1 group. This alternative method needs to perform at least as well as the Adelaide Criteria, and preferably better (i.e. the same or greater mutation detection with fewer probands tested). We have now compared the Adelaide Criteria with the Manchester Scoring System, using our cohort of 729 screened probands, and this comparison is presented at this meeting in a parallel poster.

Acknowledgements: We acknowledge the contribution of our co-workers in the SAFCS (Debbie, Vanessa and Sally), the South Australian Clinical Genetics Service (Eric and Liz), and the staff in the Genetic Pathology Laboratory at Flinders Medical Centre, and the Molecular Pathology Division of the Institute of Medical and Veterinary Science.

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