PROGRAMME

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Aspects of Cancer 2009 Research and Practice”

Tuesday 11th August  
FCC Day – Plantation Room

8.50 – 9.00  Welcome: Lara Lipton

FCC Session 1 – Plantation Room  
Chairperson: Clare Scott  
Chemoprevention and chemotherapy and BRCA1/2 mutation carriers

9.00 – 9.30  PARP inhibitors in the management of BRCA-associated cancer  
Peter Fong

9.30 – 9.45  Clinical considerations in the prevention and management of BRCA-associated breast cancer in Australia  
Gillian Mitchell

9.45 – 10.05  Challenges in cancer genetic counselling and testing for clinical trial eligibility  
Janet Tyler and Jess Koehler

10.05 – 10.30  Discussion

10.30 – 11.00  Morning tea

FCC Session 2 – Plantation Room  
Chairperson: Nicola Poplawski  
Parathyroid tumours and hyperparathyroidism - can immunohistochemistry guide genetic testing?

11.00 – 11.10  Familial cancer syndromes associated with hyperparathyroidism  
Nicola Poplawski

11.10 – 11.50  Parathyroid carcinoma  
Anthony Gill
11.50 – 12.00 Time to debate a proposed algorithm for genetic testing of parathyroid tumours
Nicola Poplawski

12.00 – 12.30 Discussion

12.30 – 1.30 Lunch

FCC Session 3 –Plantation Room
Chairperson: Marion Harris
A clinical overview of the effect of a BRCA mutation and prostate cancer

1.30 – 2.00 A BRCA2 mutation confers an increase risk of aggressive prostate cancer in Australia in New Zealand men: A clinical follow up study
Ivan Hoh

2.00 – 2.20 AN UPDATE ON THE IMPACT STUDY: Identification of Men with a genetic predisposition to Prostate Cancer: Targeted Screening in BRCA1/2 mutation carriers and controls
Gillian Mitchell

2.20 – 2.35 A survey of risk management advice provided to male BRCA1 and BRCA2 mutation carriers in Familial Cancer Centres across Australia and New Zealand
Maira Kentwell

2.35 – 3.00 The ABC of MEN - Attitudes, Barriers and Counselling - An exploration of barriers that block and strategies that engage men in the counselling process
Shauna Buscombe

3.00 – 3.30 Discussion

3.30 – 4.00 Afternoon Tea

FCC Session 4 –Plantation Room
Chairperson: Kathy Tucker
Von Hippel-Lindau disease (VHL)

4.00 – 4.20 A VHL overview
Kathy Tucker

4.20 – 4.50 VHL - case presentations
Judy Kirk, Lara Lipton, Nicola Poplawski, Kathy Tucker

4.50 – 5.00 Molecular testing for VHL - IMVS 2000-2009
Lesley Rawlings
5.00 – 5.20  Through the looking glass: A qualitative study exploring the psychosocial experiences and unmet needs of families affected by VHL  
Nadine Kasparian

5.20 – 5.50  Discussion

**FCC Staff - Drinks at Delegates expense – Serge Restaurant & Bar/Mantra Pool Deck**
Delegates organize own dinner

7.30 – 9.30  MAWSON Discussion group/Boardroom
Chairperson: Graeme Suthers

MAWSON – an online repository of genetic data to aid reporting of medical genetic tests
Wednesday 12th August

**ABCFS, ACCFS, kConFab and AOCS – Plantation Room**

8.30 – 9.00  Introduction
*Cancer Genetics, Past Present and Future - 15 Years of Progress*
Chairperson: Judy Kirk & David Goldgar

**Session 1 –**  Plantation Room
Chairperson: John Hopper

9.00 – 9.40  Advances in Breast Cancer Risk Assessment, Screening and Prevention: Applying the Lessons to an Underserved Latina Population
*Jeffrey Weitzel*

9.40 – 10.00  Aberrant luminal progenitors as the likely target population for basal tumour development in *BRCA1* mutation carriers
*Geoff Lindeman*

10.00 – 10.30  The 1000 Genomes Project: A preliminary look at what it means for future association studies.
*David J. Duggan*

10.30 – 11.00  Morning Tea

11.00 – 11.30  Inaugural Jeremy Jass Memorial Lecture – Introduction & Chair:
*Joanne Young*

Presented by Susan Parry
*Hyperplastic Polyposis – a Gastroenterologists perspective*

**Session 2 –**  Plantation Room
Chairperson: Joanne Young
Session sponsored by: VPGH Ltd

11.30 – 12.10  Deletion of the 3’ exons of *epcam (tacstd1)* causes hereditary nonpolyposis colorectal cancer with *msh2* gene promoter methylation
*Suet Yi Leung*

12.10 – 12.30  Comparing models used to predict MLH1, MSH2 and MSH6 mutation carriers: pros, cons and pitfalls
*Will Foukes*

12.30 – 12.45  Linkage to chromosome 2q32.2-q35 in families with serrated neoplasia
*Aedan Roberts*
12.45 – 1.00 Germline MUTYH Mutations in Patients with Hyperplastic Polyposis Syndrome
Daniel D Buchanan

1.00 – 1.20 Lynch syndrome cancer risks for MSH6 mutation carriers
Mark Jenkins

1.20 – 2.20 Lunch

Session 3 – Plantation Room
Chairperson: Stephen Fox

2.20 – 3.00 Pathological features of endometrial cancer in patients with Lynch syndrome: implications for genetic testing
Jose Palacios

3.00 – 3.20 Adenomas in Lynch Syndrome: Diagnostically Useful?
Michael Walsh

3.20 – 3.40 Intra-familial concordance in breast cancer pathology as an indicator of genotype in familial breast cancer
Rosemary Balleine

3.40 – 4.10 Afternoon Tea

Session 4 – Plantation Room
Chairperson: David Bowtell

4.10 – 4.40 Stromal-epithelial interactions in ovarian cancer: implications for biology and treatment
Michael Birrer

4.40 – 5.00 Are there any more ovarian tumour suppressor genes? A new perspective using ultra high resolution copy number and loss of heterozygosiy analysis
Kylie L. Gorringe

5.00 – 5.20 Somatic Genomic Alterations and Expression Profiling Link STAT3 Oncogenic Pathways to Ovarian Clear Cell Carcinoma
Michael S Anglesio

5.20 – 5.40 Genome-wide LOH and haplotype analyses of BRCAx tumours identify candidate regions for high risk breast cancer susceptibility
Ella Thompson

Delegates Organise their own Dinner

7.30 - 10.30 Poster Session + Wine and Cheese in the main foyer
## Session 5 - Plantation Room
### New strategies in providing cancer genetic counselling
**Chairperson:** Margaret Gleeson

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<tr>
<td>9.00</td>
<td>As genetics moves into mainstream medicine: challenges facing genetic counselors</td>
<td>Barbara Biesceker</td>
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<td>9.30</td>
<td>Delivering genetic counselling via telehealth: an exploration of practitioners’ and patients’ experience of a virtual consultation</td>
<td>Elvira Zilliacus</td>
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<td>9.55</td>
<td>Telephone clinics: an alternative method of delivering cancer genetic counselling services</td>
<td>Linda Cicciarelli</td>
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### Updated Familial Cancer Resources for Consumers: An evidence based approach
**Speaker:** Kate Dunlop

**10.30 - 11.00** Morning tea

## Session 6 – Plantation Room
**Chairperson:** Amanda Spurdle

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<tr>
<td>11.00</td>
<td>Genome wide association studies to identify genetic risk factors for breast cancer and genetic modifiers of breast and ovarian cancer risk in BRCA1 and BRCA2 mutation carriers</td>
<td>Fergus Couch</td>
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<td>11.40</td>
<td>DNA methylome of familial breast cancer identifies distinct profiles defined by mutation status</td>
<td>Sibylle Kugler</td>
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<td>12.00</td>
<td>Evidence for SMAD3 as a modifier of breast cancer risk in BRCA2 mutation carriers but not BRCA1 carriers</td>
<td>Logan C Walker</td>
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<tr>
<td>12.20</td>
<td>From tumour-type to genotype: a new paradigm for breast and colorectal cancer genetics services that will save lives</td>
<td>John L. Hopper</td>
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12.40 - 2.00  Lunch

Session 7-  Pavilion Room
Chairperson: Gillian Mitchell

2.00 – 2.20  Germline studies in sarcomas
David Thomas

2.20 – 2.40  Cancer treatment and prevention by reversal of the glycolytic phenotype in a model of Li-Fraumeni syndrome
Anneke Blackburn

2.40 – 3.00  An early audit of the utility of BRAF testing in 3 Victorian FCCs
Marion Harris

3.00 – 3.20  Screening and counselling issues for members of families at high-risk of pancreatic cancer
Michelle Howson

3.30 – 4.00  Afternoon tea

4.00 – 5.30  Plantation Room
OncoCARTA™ – Comprehensive Oncogene Mutation Screening
Darryl Irwin PhD, Manager - Applications and Technology, Asia Pacific, Sequenom Inc

7.30 – 10.30 Conference Dinner @ “Peppers Ballroom” (an easy walk across the road to Peppers Salt Resort & Spa Kingscliff)
### Session 8a – Plantation Room
**Chairperson: Melissa Southey**

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<tr>
<td>9.00 – 9.40</td>
<td>Genome-Wide Association Study Identifies A Novel Ovarian Cancer Susceptibility Locus On 9p22.2</td>
<td>Simon Gayther</td>
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<td>9.40 – 10.00</td>
<td>Distribution of breast cancer associated SNPs in cases and controls from the Australia Breast Cancer Family Study (ABCFS)</td>
<td>Wee Loon Ong</td>
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<td>10.00 – 10.20</td>
<td>The use of predictive or prognostic genetic biomarkers in endometrial and other hormone related cancers: Justification for extensive candidate gene SNP studies of the matrix metalloproteinase family and their inhibitors in the era of genome-wide association studies</td>
<td>Tracy A. O’Mara</td>
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### Session 8b – Pavillion Room
**Chairperson: Melanie Price**

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<td>9.00 – 9.30</td>
<td>Adaptation to living with a familial cancer or cancer risk</td>
<td>Barbara Biesceker</td>
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<td>9.30 – 9.50</td>
<td>A qualitative study of how women at high but unexplained familial risk of breast cancer perceive their risk</td>
<td>Louise Keogh</td>
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<td>9.50 – 10.10</td>
<td>A Long term Follow Up Program for BRCA1/2 and MMR Gene Mutation Carriers Practicalities of developing and running a program and initial results.</td>
<td>Lucinda Hossack</td>
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<td>10.10 – 10.30</td>
<td>Non-acceptance of an appointment at the familial cancer clinic: a preliminary telephone survey.</td>
<td>Kate Ryan</td>
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### Session 9 – Plantation Room
**Chairperson: Georgia Chenevix-Trench**

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<tr>
<td>10.50 – 11.10</td>
<td>A tale of two BRCA1 sequence variants: Evidence for intermediate function and intermediate cancer risk.</td>
<td>Amanda Spurdle</td>
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“Familial Cancer 2009: Research and Practice”
11.10 – 11.30 Rare, evolutionarily unlikely missense substitutions in *ATM* confer increased risk of breast cancer  
 **Sue Healey**

11.30 – 11.50 Assigning breast cancer risks to women with unclassified variants in *BRCA1*  
 **James Dowty**

11.50 – 12.10 Detectable methylation of the *Brcal* promoter region in peripheral blood is associated with risk of early-onset breast cancer with brcal mutation associated tumour morphology  
 **Ee Ming Wong**

**End of Meeting:** Lunch will be served
Programme

Tuesday 11th August

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“Familial Cancer 2009: Research and Practice”

FCC Session 1: Plantation Room

Chairperson: Clare Scott
PARP INHIBITORS IN THE MANAGEMENT OF BRCA-ASSOCIATED CANCER

Peter Fong
DDU and ATS Team, Royal Marsden Hospital and the Institute of Cancer Research, Address: Drug Development Unit, Royal Marsden Hospital, Sycamore House, Downs Road, Sutton SM2 5PT, London, United Kingdom.

Breast or ovarian cancer cells, which have impaired DNA repair by Homologous Recombination (HR), are highly sensitive to treatment with Parp Inhibitors. Parp 1 is a critical enzyme for Base Excision Repair (BER), binds to single strand breaks and facilitates binding of DNA repair molecules and subsequent repair of DNA. In the absence of BER, these single strand breaks become double strand DNA breaks, requiring high fidelity repair by HR. If HR is compromised, the outcome is genetic instability and death of the cancer cell. Clinical trials are underway targeting cancers that are known to have defective HR. The best example is breast or ovarian cancer in women who carry a mutation in either BRCA1 or BRCA2, the breast and ovarian cancer susceptibility genes. The Phase I trial of Olaparib/AZD2281 included patients with BRCA1/2 metastatic ovarian cancer (with a median of 3 prior treatment regimens) and showed a 63% clinical benefit rate and details will be presented. Phase II clinical trials in BRCA1/2-associated breast cancer and ovarian cancer, recently reported at ASCO 2009 will also be discussed, as will the potential role of parp inhibition in sporadic breast and ovarian cancer. Thus, by first studying a defined genetic context, the synthetic lethal combination of underlying impaired HR and drug-induced impaired BER in tumour cells has been designed, explored and achieved exciting preliminary clinical results.
Breast cancer is the most common cancer associated with the inheritance of a germline BRCA1 or BRCA2 mutation. Providing advice about the management of this cancer risk is therefore an important role for Familial Cancer Centres.

During the presentation we will present:

- A summary of the current chemoprevention options available to BRCA mutation carriers
- A summary of the uptake of preventive and/or therapeutic mastectomy in BRCA mutation carriers and the role of BRCA mutation testing around the time of cancer diagnosis
- A summary of the Australian contribution to the PARP inhibitor studies to date and the current “state of play” regarding current and planned PARP inhibitor trials in Australia.
- A summary of the role of platinum agents in the treatment of BRCA-associated breast cancer including an update about the current recruitment and future plans for the “BRCA Trial”
CHALLENGES IN CANCER GENETIC COUNSELLING AND TESTING FOR CLINICAL TRIAL ELIGIBILITY

Jessica Koehler, Janet Tyler and Sian Greening
Hereditary Cancer Clinic, Prince of Wales Hospital

The first reports of possible targeted therapies for BRCA1/2 mutation carriers using PARP inhibitors were published in 2005 (Bryant et al) and phase 2 clinical trials began in 2007. An increasing number of patients with metastatic breast or ovarian cancer are now undergoing BRCA1/2 testing to determine trial eligibility. This presents a new clinical scenario where genetic testing impacts the treatment options available to the patient.

To establish eligibility for the PARP inhibitor trial, twelve women with ovarian cancer and five with breast cancer have undergone genetic testing. 4/17 individuals had no family history of breast or ovarian cancer and 2/16 were from families with a known BRCA mutation. Five BRCA1 mutations and one BRCA2 mutation (6/17) were identified.

Each patient presented with unique issues and providing genetic counselling in this setting raised a number of new practical, ethical and psychosocial challenges. The following psychosocial issues arose in many of the cases:

- Unrealistic hopes and expectations of genetic testing results;
- Uptake of genetic counselling testing that had previously been declined;
- Pressure to undergo genetic testing;
- Frustration and disappointment over outcomes;
- Downplay of the family implications of genetic testing;
- Genetic testing at the end stage of disease.

Common practical considerations included:

- Liaison with referring doctors;
- Timing constraints of trial period, patient health and genetic testing turnaround;
- Eligibility for publicly funded genetic testing;
- Trial eligibility issues;
- Results interpretation and results giving.

Strategies for genetic counselling in this situation will be discussed and include being realistic about the likely results, managing patient expectations and discussing the family implications of results at each stage of counselling.

Bryant H.E. et al “Specific killing of BRCA2 deficient tumours with inhibitors or Poly(ADP-Ribose) Polymerase” 2005 Nature 434:913-917
Programme

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“Familial Cancer 2009: Research and Practice”

FCC Session 2: Plantation Room

Chairperson: Nicola Poplawski
FAMILIAL CANCER SYNDROMES ASSOCIATED WITH HYPERPARATHYROIDISM

NK Poplawski

Familial Cancer Unit, Women's and Children's Hospital, North Adelaide, South Australia, Australia
University of Adelaide, Adelaide, Australia
Email: nicola.poplawski@cywhs.sa.gov.au

Primary hyperparathyroidism is a common endocrine disorder, with a population prevalence of approximately 3/1000.¹ Excluding individuals who have renal failure or are part of a known or suspected MEN1 family, the most common cause of primary hyperparathyroidism is a single parathyroid adenoma (90-95%). Most of the other cases are explained by multi-glandular hyperplasia (~5%) or multiple adenomata (2-3%).² ³ Parathyroid carcinoma is uncommon, accounting for less than 1% of cases.²

Most parathyroid tumours are sporadic, however a small proportion (5-10%) are associated with familial disorders. Although the underlying genetic cause of familial parathyroid tumours is able to be identified in a proportion of cases, the genetic basis of many familial tumours remains unknown. Disorders associated with hyperparathyroidism include:
• multiple endocrine neoplasia type 1 (MEN1), caused by mutations in the MEN1 gene
• multiple endocrine neoplasia type 2A (MEN2A), caused by mutations in the RET gene
• hyperparathyroidism-jaw tumour syndrome (HPT-JT), caused by mutations in the CDC73 gene (aka the HRPT2 gene)
• familial hypocalciuric hypercalcaemia (FHH), caused by mutations in the CASR gene
• familial isolated hyperparathyroidism (FIHP), caused by mutations in a number of genes including MEN1, RET and CDC73, as well as one or more currently unidentified genes

In this short paper I will provide a brief overview of the clinical features of these syndromes, with emphasis on hyperparathyroidism and parathyroid tumours.

PARATHYROID CARCINOMA
Anthony Gill

Historically it has been extremely difficult if not impossible to make a confident clinical or pathological diagnosis of parathyroid carcinoma prospectively without unequivocal evidence of metastatic disease. Although more accurate than clinical findings, the histopathological diagnosis of parathyroid carcinoma is very imperfect. Currently the pathological diagnosis of parathyroid carcinoma is restricted to lesions which show unequivocal invasive growth as evidence by perineural invasion, full thickness capsular penetration with growth into adjacent tissues or metastasis. Features such as an abnormal architecture with broad bands of intraparathyroidal and peritumoural fibrosis, profound cytological atypia, a trabecular growth pattern, invasion into the capsule of the parathyroid, a high mitotic rate, tumour necrosis and local recurrence are useful in supporting a diagnosis of parathyroid carcinoma but also occur not uncommonly in benign adenomas.

Although it is confounded by the fact that surgical excision, particularly radical or en bloc excision, has the capacity to interrupt the natural history of parathyroid carcinoma and that late recurrence is not uncommon, perhaps as many as 50% of biologically malignant parathyroid carcinomas will be considered benign using conventional clinicopathological criteria and perhaps as few as 15% of all cases diagnosed as carcinoma prospectively (36% if rigid histopathological criteria for malignancy are applied by a specialist endocrine pathologist) will go on to behave in a malignant manner.

The subjectivity and inadequacy of the pathological diagnosis of parathyroid carcinoma is further complicated by the geographic and institutional variation in incidence. In most series carcinoma accounts for considerably less than 1% of all patients with primary hyperparathyroidism but this figure is as high as 5% in some Japanese and Italian reports. Of course specific environmental or genetic factors may account for this difference, but it is intriguing to postulate that different pathological thresholds for diagnosis play a role.

In recent years there has been a quantum leap in the understanding of the molecular pathway associated with parathyroid carcinogenesis and its relationship to the previously obscure Hyperparathyroidism Jaw Tumor (HPT-JT) syndrome [OMIM#145001]. In addition to hyperparathyroidism, patients with the autosomal dominant HPT-JT syndrome are at risk of developing renal lesions and fibro-osseous jaw tumors. The gene responsible for HPT-JT, named HRPT2 for “Hyperparathyroidism 2”, was recently described and maps to 1q25-q31.17 HRPT2 contains 17 exons and encodes a 531 amino acid protein termed parafibromin because of its relationship to parathyroid disease and fibro-osseous lesions.

Although sporadic parathyroid carcinoma is rare, in HPT-JT syndrome a striking 15% of all parathyroid lesions may be malignant. Therefore it was not surprising that after germline HRPT2 mutations were found in kindreds with HPT-JT, HRPT2 somatic mutations were described in large numbers of sporadic parathyroid carcinomas. The combined results of several studies indicate that inactivating mutations of HRPT2 are found in approximately 70% of all parathyroid carcinomas. The intimate relationship between parathyroid carcinoma and HPT-JT syndrome is further illustrated by the somewhat unexpected finding that up to 20% of patients with apparently sporadic parathyroid carcinoma actually harbor a germline mutation of HRPT2 and therefore have unrecognized HPT-JT. It is therefore recommended that all patients with parathyroid carcinoma be screened for HPT-JT.

Molecular analysis of HRPT2 is therefore a validated approach for the diagnosis of both HPT-JT and sporadic parathyroid carcinoma. However the time and resources for molecular analysis are beyond the means of most surgical pathology laboratories. For this reason,
immunohistochemistry for parafibromin has been proposed as a quick, cheap and efficient method of assessing HRPT2 mutation and/or allelic loss status.

We demonstrated completely negative parafibromin staining in 8 of 11 apparently sporadic parathyroid carcinomas (defined as not only meeting the WHO criteria for malignancy but also behaving in a malignant manner) and 3 of 4 HPT-JT related parathyroid tumours, but positive staining in 100 unselected adenomas and 56 of 57 “giant adenomas” (defined as adenomas greater than 2g). The one giant adenoma which showed negative staining for parafibromin was initially classified as “atypical adenoma” and it is reasonable to suggest that it represents an occult parathyroid carcinoma.

There are some inherent technical difficulties in using absence of staining for an antibody as a criteria for diagnosis and we emphasize that for a parathyroid tumour to be considered negative for parafibromin, there should be a strong positive internal control (both thyroid and non-neoplastic parathyroid are satisfactory) and there should be sufficient antigen retrieval so that endothelial cells stain positively immediately adjacent to the negative tumour cells. This approach is very similar to that used to assess microsatellite instability in colorectal tumours using MSH2 and MLH1 immunohistochemistry and the antibody is equally capricious. We now do not think that focal or patchy negative staining for parafibromin is significant.

Given the difficulties with parafibromin immunohistochemistry, we have recently investigated PGP9.5 as a surrogate marker for the HRPT2 mutated phenotype. We have demonstrated that diffuse strong staining for PGP9.5, which we arbitrarily defined as staining of more than 50% of tumour cells, is highly correlated with negative parafibromin staining, HRPT2 mutation and therefore the malignant/HPTJT phenotype. Furthermore PGP9.5 appears to be positive in the very occasional instances where there is a point mutation of HRPT2 (which may be associated with positive staining for parafibromin).

We have therefore proposed that all clinically or histologically atypical parathyroid tumours undergo parafibromin and PGP9.5 immunohistochemistry. If parafibromin is negative or PGP9.5 is strongly positive we diagnose malignancy and offer HRPT2 germline testing. Because only 70% of biologically malignant parathyroid tumours are HRPT2 mutated, neither immunohistochemistry nor DNA testing can be used to exclude a diagnosis of carcinoma. However we do not usually offer genetic testing for HRPT2 if the tumour is parafibromin positive and PGP9.5 negative on the basis that this makes germline HRPT2 mutation extremely unlikely.

Acknowledgements:
Deborah Marsh, Viive Howell Kolling Institute of Medical Research
TIME TO DEBATE A PROPOSED ALGORITHM FOR GENETIC TESTING OF PARATHYROID TUMOURS

NK Poplawski¹, M Burt² and A Gill³

1. Familial Cancer Unit, Women's and Children's Hospital, and University of Adelaide, Adelaide, South Australia
2. Southern Adelaide Diabetes and Endocrine Services, Flinders Medical Centre and School of Medicine, Flinders University, Adelaide, South Australia
3. Dept of Anatomical Pathology, Royal North Shore Hospital and University of Sydney, Sydney, New South Wales

Following oral presentations by Nicola Poplawski and Anthony Gill, the following algorithm for genetic testing in individuals with parathyroid tumours will be tabled for discussion.

[Diagram of the algorithm for genetic testing of parathyroid tumours]

- **Hyperparathyroidism due to parathyroid carcinoma / adenoma / hyperplasia**
  - **Review histology**
  - **Parathyroid carcinoma**
  - **Parathyroid adenoma or hyperplasia**
    - **Assess for other clinical features and take a family history**
      - **Clinical features and/or family history suggests a syndrome diagnosis**
        - **Is there**
          - A family history of parathyroid tumours
          - Onset <30 years
          - Multiple adenomas
          - Atypical histology
          - Recurrent adenoma
        - **Test appropriate gene(s)**
          - CDC73 / MEN1 / CASR / RET
        - **Yes**
          - Paraffinum immunohistochemistry
            - Normal
              - Sequentially screen
                1. MEN1
                2. CDC73
                3. CASR*  
          - Abnormal
            - Sequentially screen
              1. CDC73
              2. MEN1
              3. CASR*
        - **No**
          - Sporadic hyperparathyroidism
            - Genetic testing not indicated in most cases
              - FCU team happy to review on specific request

*CASR: indicated in selected cases where a positive/negative test would influence management
Programme

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“Familial Cancer 2009: Research and Practice”

FCC Session 3: Plantation Room

Chairperson: Marion Harris
BRCA2 MUTATION CONFERS AN INCREASED RISK OF AGGRESSIVE PROSTATE CANCER IN AUSTRALIA AND NEW ZEALAND

Ivan MY Hoh1,2, HJ Thorne2, AJ Willemse2, J Li3, JL Hopper3, SB Fox2, J Sambrook2, DM Bolton1, David Clouston3 and the kConFab consortium

1Department of Urology, Austin Health, Melbourne, Australia; 2Peter MacCallum Cancer Centre, Melbourne, Australia; 3Centre for Molecular, Environmental, Genetic and Analytical Epidemiology, University of Melbourne, Victoria, Australia; 3Department of Pathology, Austin Health, Melbourne, Australia.

Introduction and Objective

Certain germline mutations in the breast cancer predisposition genes, in particular BRCA2, have been implicated in a greater susceptibility to prostate cancer. This study aims to determine the clinical significance of BRCA2 mutation in men with prostate cancer in breast cancer families in Australia and New Zealand.

Materials and Methods

From the kConFab consortium database of 1300 families, 840 patients with prostate cancer were identified and the BRCA mutation status confirmed on 167 participants using sequencing or MLPA analysis. Clinical details were collected retrospectively and the pathology reports were updated using the 2002 TNM staging. The total duration of follow-up was 21.5 years (1981 – 2009). Curative treatment was defined as either surgery or radiotherapy.

Results

There were 35 BRCA2 patients in this study. The median age at diagnosis was 65.5 years old (range 43 – 77). The median ages at deaths were 72 years. Out of a total of 19 deaths, 16 were prostate related. The mean duration from diagnosis to death were 4.8 years (range 1-12 years). Treatment data were unknown in 5 patients and were therefore excluded from the analysis.

20 patients (69%) received curative treatment i.e. either surgery (n=9) or radiotherapy (n=11). Within this group, a total of 9 patients had a prostate related death, of whom, 6 had radiotherapy and 3 had surgery. The mean duration of survival between surgery and radiotherapy were 5 and 8.3 years (p= 0.12). When comparing those who received curative vs. non-curative treatments, the mean duration of survival is 7.2 vs. 2.6 years (p= 0.0033).

Conclusions

Prostate cancer in men with a family history of breast cancer, and who have been shown to be positive for the BRCA2 gene, appears to have a more aggressive a natural history than prostate cancer in the general population. Affected men appear to be younger at time of disease diagnosis, have a higher disease specific mortality and a younger age of disease related mortality. A significant number of BRCA2 prostate cancer patients have advanced disease unsuitable for potentially curative treatment at the time of diagnosis, resulting in a mean survival after diagnosis in this of 4.8 years only. Further evaluation is required but being a BRCA2 gene carrier appears to be associated with a significantly worse prognosis for men with prostate cancer. This will impact upon potential approaches to screening for and treatment of prostate cancer.
AN UPDATE ON THE IMPACT STUDY: IDENTIFICATION OF MEN WITH A GENETIC PREDISPOSITION TO PROSTATE CANCER: TARGETED SCREENING IN BRCA1/2 MUTATION CARRIERS AND CONTROLS


Background: IMPACT is an international study that was set up to investigate targeted prostate cancer screening with serum PSA in men at increased risk of developing prostate cancer due to a BRCA1 or BRCA2 gene mutation (BRCA2=RR 4.65; BRCA1=RR 1.07).

Aims: The immediate goal of the study is to determine the incidence of raised serum prostate specific antigen (PSA) and abnormal prostatic biopsy in this high-risk group and hence the sensitivity and specificity of targeted prostate cancer screening with PSA. Additional aims include the opportunity to develop new markers of prostate cancer and/or prostate cancer predisposition genes by serially banking serum, plasma and urine and to determine if the pathology of prostate cancer detected by targeted screening in BRCA1/2 mutation-carriers differs from that in controls.

Method: Men aged 40-69 years testing positive (study group) or negative (control group) for a known familial BRCA1 or BRCA2 gene mutations are eligible. Serum PSA samples are collected yearly for five years, in addition to yearly blood and urine samples (which are banked for future translational biomarker studies). An elevated PSA >3 ng/ml triggers a urological referral for transrectal ultrasound and biopsy. Subsequent clinical management depends upon the biopsy results. Two additional prostate cores are taken for banking within IMPACT for future translational studies.

Progress: At May 2009, 665 men from 29 centres across 10 countries had been recruited (BRCA1=258, 40%; BRCA2=205, 29%; Controls=192, 29%), with the overall aim to enrol 850 carriers and 850 controls. Australia’s contribution makes up a quarter of the cohort, having recruited 174 of these men from 7 centres in Victoria, NSW, SA and WA. (Centres in Tasmania and QLD are due to start recruiting this year).

Discussion: In a recent paper by Mitra et al (2008)* the histological characteristics of prostate cancers in BRCA1/2 mutation carriers (that included one UK IMPACT participant) were reviewed and compared to a control group. They found that carriers had a significantly higher Gleason score and concluded that having a BRCA1/2 mutation resulted in more aggressive disease. Unpublished data on prostate cancers found in men from the IMPACT study to date support this finding and show a trend toward higher Gleason scores in BRCA2 mutation carriers and higher stage cancers in both BRCA2 and BRCA1 mutation carriers.

Conclusion: Currently there are limited options available to male BRCA1/2 mutation-carriers to manage their increased prostate cancer risk. It is too early to tell from the IMPACT study if PSA screening is an effective way to screen for prostate cancer in this group. However, with new data emerging to indicate that carriers who develop prostate cancer will also have more aggressive disease, it is important that the IMPACT study continues as planned so we can provide them with evidence based guidelines on how to manage their risk as well as discovering additional prostate cancer markers to improve the utility of prostate cancer screening.

A SURVEY OF RISK MANAGEMENT ADVICE PROVIDED TO MALE BRCA1 AND BRCA2 MUTATION CARRIERS IN FAMILIAL CANCER CENTRES ACROSS AUSTRALIA AND NEW ZEALAND

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Men’s health is increasingly being recognized as an important specialty area of health promotion and clinical practice, reflecting an emerging international men’s health movement. This is particularly relevant to the Familial Cancer setting, as men who inherit BRCA1 or BRCA2 mutations are at heightened risk of developing prostate (and breast) cancer. In addition, male BRCA1/2 mutation carriers may be at increased risk of developing other malignancies, including pancreatic cancer, biliary tract cancer and malignant melanoma.

Research has suggested men who have a family history of prostate cancer desire more information about personal risk and risk management options, with a demonstrated willingness to engage in preventative practices (1, 2). Even if men’s experience of cancer in the family related to breast/ovarian cancer, men have been shown to harbor fears of developing cancer themselves. It has been suggested that men have a functional view of their bodies and see health care as a ‘fit-it’ cure, and therefore may respond better to health care interventions that offer tests, facts and figures, mirroring an action-oriented approach. In the context of Familial Cancer, the multi-disciplinary Genetic Counselling setting represents an ideal setting in which to address the informational (as well as emotional and behavioural) needs of men. However, the precise cancer risks faced by men are either poorly understood or not well recognised. As a result, current clinical practice guidelines for men harboring BRCA1/2 mutations are not well defined.

The purpose of this survey was to document the cancer risk and clinical management advice currently provided to male BRCA1 or BRCA2 mutation carriers in Familial Cancer Centres across Australia and New Zealand. Questionnaires were sent to 19 clinics, in order to ascertain the general practices in place by genetic counsellors and clinicians at each clinic. The results of the survey will be presented for general discussion. It is hoped that the findings will form the basis for generating consistent ‘best practice’ guidelines for men who have inherited a BRCA1/2 mutation.

THE ABC OF MEN - ATTITUDES, BARRIERS AND COUNSELLING - AN EXPLORATION OF BARRIERS THAT BLOCK AND STRATEGIES THAT ENGAGE MEN IN THE COUNSELLING PROCESS.

Shauna Buscombe
Family Cancer Clinic, Peter MacCallum Cancer Centre

Genetic testing and screening for prostate cancer risk will eventually move from research to the clinical setting as has been the case with breast cancer and colorectal cancer. Yet historically we know that men have a lower uptake of genetic services than women.

- What factors influence men’s decision to attend?
- What do men think about when they hear the word “counselling”?
- What are the attitudinal and social factors that might deter men from attending?
- How can genetic counsellors, the majority of whom are women, better recognize, understand and address men’s concerns and fears in relation to the genetic counseling process?
- How might we harness men’s motivation so that they do attend and ultimately benefit from the process?

This presentation will specifically explore the needs of men in the counselling process and attempt to identify strategies which might encourage men’s active engagement in the genetic counselling process.
Programme

Tuesday 11 August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

FCC Session 4: Plantation Room

Chairperson: Kathy Tucker
A VHL Overview

Kathy Tucker: Prince of Wales Hospital, Sydney
VHL Case Presentations

Judy Kirk, Lara Lipton, Nicola Poplawski, Kathy Tucker
von Hippel-Lindau disease (VHL) is a rare, genetic multi-system disorder characterized by the abnormal growth of tumours and blood vessels in the central nervous system, brain, the retina of the eyes, the adrenal glands, kidneys or pancreas. The disease is caused by mutations in the VHL tumour suppressor gene located on chromosome 3 and is autosomal dominantly inherited.

Molecular testing to determine mutations in the VHL gene commenced at the IMVS in November, 2000 with a single South Australian proband, followed by predictive testing for this family. In 2008, thirty seven gene screens and twelve presymptomatic tests were performed on samples received from SA, interstate and New Zealand. To date, thirty-four positive probands and twenty-six positive presymptomatic cases have been identified. This presentation details the mutations detected in these samples.
THROUGH THE LOOKING GLASS: A QUALITATIVE STUDY
EXPLORING THE PSYCHOSOCIAL EXPERIENCES AND UNMET NEEDS
OF FAMILIES AFFECTED BY VHL

Nadine Kasparian¹,², Alison Rutstein², Janet Tyler³, Jessica Koehler³ and Kathy Tucker³.

1) School of Women’s and Children’s Health, Faculty of Medicine, University of New South Wales, Kensington, NSW, Australia;
2) Psychosocial Research Group, Department of Medical Oncology, Prince of Wales Hospital, Randwick, NSW, Australia;
3) Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick, NSW, Australia.

From a medical perspective, surveillance for tumours associated with Von Hippel-Lindau disease (VHL) is complex, involves monitoring multiple organ systems over a lifetime, and is an important means of tumour control. Nevertheless, tumour control is only the first step and ensuring quality of life for the patients and families affected by VHL requires careful consideration. Despite well-established protocols for the medical management of VHL, families affected by this rare tumour syndrome continue to face numerous psychosocial challenges. The primary aim of this study was to qualitatively explore the major psychological, social and behavioural problems faced by families affected by VHL. For this purpose, a semi-structured individual interview was developed to explore patients’ and carers’ experiences of VHL along several life domains, including: interpersonal relationships, education and career opportunities, family communication, physical health and emotional well-being, as well as unmet information and supportive care needs relating to VHL. Participants were ascertained via the Hereditary Cancer Clinic at the Prince of Wales Hospital in Sydney, Australia, and purposive sampling was used to explore experiences from as many different perspectives as possible. A total of 23 individual interviews were conducted over the telephone with 15 patients and 8 carers (age 18-75 years), yielding a response rate of 75%. Overall, a diverse range of experiences were reported, with many participants describing periods of social isolation; limited access to, or awareness of, support services; feelings of transmission guilt; and limited life opportunities. A number of carers spoke of the challenges they had encountered over the years of caring for a family member with VHL. In contrast, resilience was also a major theme, with several participants providing examples of the positive coping strategies that they had developed as a result of their experiences. Moreover, all participants voiced overwhelming support for continued efforts to improve supportive care services and resources for families affected by VHL. The findings of this study will be used to inform the planning and development of a larger international collaborative study to examine the prevalence and correlates of psychological distress, as well as resilience, among individuals affected by VHL.
MAWSON – AN ONLINE REPOSITORY OF GENETIC DATA TO AID REPORTING OF MEDICAL GENETIC TESTS

Markus Stumptner, & Paula Swatman, School of Computer & Information Science, University of South Australia; Graeme Suthers & Scott Grist, SA Pathology, Adelaide SA.

Genetic testing in familial cancer is new and effective. Relatives with the mutation can have targeted surveillance, and those without can be spared it. The initial step of finding the mutation is expensive, and is often difficult to interpret. International databases can assist in interpreting these findings, but the vast bulk of information from diagnostic laboratories is never shared. The MAWSON database is an online repository of genetic data that is collected semi-automatically from laboratories. Laboratories can review these data to improve the quality of test interpretation, thereby reducing uninformative testing. MAWSON also ensures national consistency in reporting. These data can be used to monitor the quality of genetic testing, and the collated data can be readily submitted to international databases. While this model is being developed in the context of familial cancer, it would be immediately applicable in all areas of genetic testing both in Australia and internationally. This project is funded by the Federal Department of Health & Ageing, and is in collaboration with the Australian node of the Human Variome Project. This workshop will be an opportunity for clinicians and medical scientists to hear about progress and to suggest strategies for dealing with specific issues.
Programme

Wednesday 12 August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 1: Plantation Room

Chairperson: John Hopper
ADVANCES IN BREAST CANCER RISK ASSESSMENT, SCREENING & PREVENTION: APPLYING THE LESSONS TO AN UNDERSERVED LATINA POPULATION

Jeffrey N. Weitzel, MD
1) Department of Clinical Cancer Genetics, City of Hope Cancer Center, Duarte, CA; 2) Olive View Medical Center, Sylmar, CA; 3) USC County Medical Center; 4) Department of Psychology, City of Hope Cancer Center, Duarte, CA; 5) Columbia University, NY.

Breast cancer (BC) is the most common cancer and the leading cause of cancer death in Latina women. Mutations in BRCA genes are associated with 5% of all BC and a larger proportion of young women with BC. We previously reported on the prevalence of deleterious BRCA mutations (31% of 110 families) in a Latina high-risk clinic in Los Angeles, and also identified a unique recurrent large BRCA1 rearrangement (deletion of BRCA1 exons 9-12). BRCA1 185delAG was detected in several independent Latina families, and through haplotyping we established that they shared a common ancestral origin with Jewish carrier families. The presence of additional recurrent mutations suggested possible founder effects in this population of largely Mexican ancestry.

A Strategy for More Efficient BRCA Analysis in an Underserved Latina Population

We hypothesized that a panel of recurrent Latina BRCA mutations to pre-screen high-risk patient samples will demonstrate clinical utility and reduce genotyping cost. Using a new high-throughput Sequenom® platform, we developed a prototype multiplex panel to test for recurrent BRCA mutations and a 3-primer assay to test for the BRCA1 rearrangement mutation. We created a clinical protocol and procedure that enabled sample collection, DNA extraction, amplification and mutation panel analysis on the Sequenom platform within a 72 hour time frame. We piloted this 18 mutation panel in the clinical genetic cancer risk assessment setting. Positive assays were confirmed in a CLIA-approved laboratory by sequencing of the specific segment, and comprehensive BRCA sequencing was performed on all samples with negative results. We applied the panel prospectively in a pilot study of 23 consecutive Latina breast cancer patients referred to the City of Hope Cancer Screening & Prevention Program Network for genetic cancer risk assessment. A substantial proportion (4/7, 57%) of deleterious BRCA mutations were detected by the panel in this proof of principle pilot study in our high risk clinic, suggesting strong translational potential.

Impact of Genetic Cancer Risk Assessment on Cancer Screening and Prevention Behaviors in an Underserved Predominantly Latina Population

Genetic cancer risk assessment (GCRA) has the potential to identify persons at increased risk for cancer prior to the onset of disease, when early detection or prevention strategies would be most effective. Despite clinical studies documenting the efficacy of cancer risk reduction measures in high-risk individuals, low-income, underinsured or ethnic minority individuals have a disproportionate burden of cancer and limited access to GCRA. Methods: City of Hope Cancer Screening & Prevention Program Network outreach clinics were established in two regional indigent healthcare delivery systems, QueensCare Health & Faith Partnership and Olive View Medical Center. One hundred thirty-two of 153 (86%) probands referred for GCRA attended their scheduled consultation. All participants were consented and enrolled in an IRB-approved hereditary cancer registry at the time of their consultation. The consultation was conducted by an experienced bilingual cancer risk counselor/physician team and risk management recommendations were given based on BRCA test results or empiric risk estimates. In addition, 42 at-risk relatives (ARR) of BRCA carriers entered study and underwent informative BRCA testing. Baseline health behaviors were obtained by survey pre-GCRA and adherence to cancer screening and prevention recommendations was assessed at least 1-year post GCRA via a mailed follow-up questionnaire. Items included current status.
of health, perceived cancer risk and risk management activities. A telephone call was made to prompt survey completion one week following the mailing. **Results:** Of 174 participants, 131 were eligible for 1-year follow-up and constitute the sample for this analysis; follow-up data was obtained on 88 (67%). All respondents were female and 84% were of Latina descent. Fifty-five (63%) had breast cancer (13 bilateral), 3 (3.4%) had ovarian cancer and 30 were unaffected. Mean age of breast cancer diagnosis was 38.4 years. Deleterious *BRCA* mutations were detected in 17/52 (33%) probands, and 11/17 (65%) ARR (from 10 families). As of 1-year post-GCRA, risk-reducing salpingo-oophorectomy (RRSO) was obtained by 11 of 18 (61%) *BRCA* carriers over age 35 who had completed childbearing, 6 of whom also obtained risk-reducing mastectomy (RRM). One additional *BRCA* carrier obtained a RRM only, for a total of 7 of 25 (28%) carriers with at-risk breast tissue electing to undergo RRM post-GCRA. Post-GCRA, of those with at-risk breast tissue and of appropriate age for screening, 53/73 (73%) were performing monthly self-breast exams, and 67/79 (85%) obtained clinical breast exams and 57/66 (86%) obtained a mammogram as recommended. Overall, more risk appropriate screening and prevention behavior was exhibited post-GCRA. The possibility of a cultural influence on risk reduction choices is evident in that fewer Latinas obtained RRSO (61% vs. 75%) compared to a reference cohort of non-Hispanic *BRCA* carriers seen for GCRA at the cancer center.

**Conclusion:** Our study indicates that underserved patients embraced the opportunity for GCRA services, attended their scheduled consultations, had a high level of participation in follow-up surveys and showed a positive impact on cancer screening and prevention behaviors.
ABERRANT LUMINAL PROGENITORS AS THE LIKELY TARGET POPULATION FOR BASAL TUMOUR DEVELOPMENT IN BRCA1 MUTATION CARRIERS

Elgene Lim1,2,*, François Vaillant1,*, Di Wu1,2, Natasha C Forrest1, Bhupinder Pal1, Adam H Hart1, Marie-Liesse Asselin-Labat1, David E Gyorki1,2, Teresa Ward1, Audrey Partanen4, Frank Feleppa4, Lily I Huschtscha5, Heather J Thorne6, kConFab7, Stephen B Fox6, Max Yan6, Juliet D French8, Melissa A Brown8, Gordon K Smyth1, Jane E Visvader1,† and Geoffrey J Lindeman1,2,4,†

1The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC. 2The University of Melbourne, Parkville, VIC. 3Monash University, Clayton, VIC. 4The Royal Melbourne Hospital, Parkville, VIC. 5Children's Medical Research Institute, Westmead, NSW. 6Peter MacCallum Cancer Centre, East Melbourne, VIC. 7The Kathleen Cunningham Consortium for Research into Familial Breast Cancer. 8The University of Queensland, Brisbane, QLD.*†Equal contribution.

Basal-like breast cancers arising in women carrying mutations in the BRCA1 gene are thought to develop from the mammary stem cell. To explore early cellular changes that occur in BRCA1 mutation carriers, we have prospectively isolated distinct epithelial subpopulations from normal breast tissue and pre-neoplastic breast tissue from BRCA1 mutation carriers. Normal breast tissue (depleted of haemopoietic and endothelial cells) was fractionated by FACS into four populations on the basis of CD49f (α6-integrin) and EpCAM expression. Basal stem/progenitor cells (CD49fhiEpCAM–) were identified by their ability to generate outgrowths following transplantation into ‘humanized’ mammary fat pads of immunocompromised mice. Committed luminal progenitors (CD49fEpCAM+) were identified on the basis of luminal marker expression and colony forming ability in a 3D in vitro assay. Similar to the mouse mammary gland, mature luminal cells (CD49f EpCAM+) expressed the highest proportion of cells positive for ER and GATA-3. A fourth population (CD49f EpCAM–) was found to contain breast stromal fibroblasts.

We next evaluated histologically normal breast tissue from healthy BRCA1 mutation carriers, obtained following prophylactic mastectomy. Unexpectedly, we found that breast tissue from BRCA1 mutation carriers harbored an expanded luminal progenitor population that consistently showed factor-independent growth in vitro. In contrast, the stem cell-containing population was reduced in BRCA1 breast tissue and did not exhibit factor-independent growth. Similar to human breast tissue, mammary cells from Brca1-deficient mice (MMTV-cre Brca1f/f), also exhibited aberrant luminal progenitor cell activity. Approximately four-fold fewer repopulating mammary stem cells were present in Brca1-deficient mice compared to normal littermate controls.

Gene profiling studies were performed to gain insights into the molecular characteristics of luminal progenitor cells and their relationships between normal mammary epithelial cells and breast cancer subtypes. This revealed that BRCA1 breast tissue and basal breast tumours were more similar to normal luminal progenitor cells than any other subset, including the stem cell-enriched population. C-KIT emerged as a key marker of luminal progenitor cells and was more highly expressed in BRCA1-associated pre-neoplastic tissue and tumours. Our findings suggest that an aberrant luminal progenitor cell population is a key target for transformation in BRCA1-associated and possibly other basal breast tumours.
THE 1000 GENOMES PROJECT: A PRELIMINARY LOOK AT WHAT IT MEANS FOR FUTURE ASSOCIATION STUDIES.

David J. Duggan, Ph.D.

Translational Genomics Research Institute (TGen), Phoenix, AZ, U.S.A.

Advances in technology usually precede advances in knowledge. In the last two decades, the genetics and genomics field has seen a rapid advance in both technology and knowledge. In the most recent 5 year time period, a completed catalog of common genetic variation, known as the HapMap, has revolutionized human genetic studies, making it possible to systematically test common DNA variants for an association to disease risk. This genome-wide resource has made possible discovery of over 200 new genes that contribute to numerous common diseases including diabetes, cardiovascular disease, inflammatory conditions, neurological disease, and cancer. While successful, HapMap has a major limitation. It contained only the most common genetic variants, those with frequencies above 5% and encompassed only single nucleotide polymorphisms (SNPs).

By making use of the latest technological advance, next generation sequencing, the 1000 Genomes Project will capture variations in the human genome’s sequence and structure at the highest resolution yet. The 1000 Genomes Project will sequence the genomes of at least 1000 people, discovering both SNPs and structural variants. The scientific goals of the Project are to produce a catalog of variants that are present at 1% or greater frequency in the human population across most of the genome, and down to 0.5% or lower within genes. This will increase the sensitivity of disease discovery efforts across the genome five-fold and within gene regions at least 10-fold. These data will have a positive and profound implication on the design and execution of future association studies including genome-wide and candidate gene and pathway studies. In this presentation, we will take a preliminary look at (1) the 1000 Genomes data, (2) it’s likely impact on genome-wide association studies, and (3) compare the cost of genotyping up to 4 million SNPs using genotyping versus next generation sequencing technologies.
Late in 2008, Jeremy Jass passed away just over a year after he was diagnosed with glioblastoma multiforme. During his illness he calmly fought against this aggressive cancer, and he never expressed a word of bitterness that life has been dealt him this terrible blow. He was just 57 years old.

For those who did not know him, Jeremy was a brilliant and innovative world-class colorectal pathologist. For over two decades, he produced important and original work in the form of several hundred papers, many books, book chapters, and lectures. Jeremy was an avid writer, and through his letters both to the editors of journals, and to many of us, he provided leadership and teaching. He combined this activity with works forming practical lasting foundations for both research as well as the doing of clinical good. Many lives have been saved by his establishment of the familial colorectal cancer registries in Queensland, Australia and Auckland, New Zealand.

Throughout his career, he enriched our understanding of colorectal cancer by encouraging the view that it was a heterogeneous group of conditions. His work on the evolution of colorectal cancer subsets has served as a basis for an expanded approach to screening, diagnosis and research, and this will live on as an important legacy. His innovative work was not without price however. He often made important observations many years before anyone else, and there were times when he endured overt hostility because of this. Time has proven him correct, and his ideas now have widespread acceptance. In the case of the serrated pathway, he published a paper challenging the dogma that all hyperplastic polyps were innocuous over 25 years ago.

He made major contributions to familial colorectal cancer in the understanding of Lynch syndrome cancers, and he also published detailed descriptions of two New Zealand families with multiple cases of serrated neoplasia in 1996 and 1997 respectively. Familial serrated neoplasia is relatively common in Australia and New Zealand, though not readily recognised, and it would be a fitting tribute if this condition, once characterised, could one day bear his name.

Jeremy was in addition to being a marvellous pathologist, a humble, kind and generous person. He willingly shared his unpublished data with other researchers, and was highly collaborative and open-minded. He remains an inspiration to all who knew him. The sad irony of his cancer was, that in affecting his brain, his condition robbed him of any chance to outsmart it. He came to us briefly, made our lives extraordinary, then moved on. This lecture is an appropriate way to honor and remember him.
Programme

Wednesday 12th

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 2: Plantation Room

Chairperson: Joanne Young

“Familial Cancer 2009: Research and Practice”
DELETION OF THE 3’ EXONS OF EPCAM (TACSTD1) CAUSES HEREDITARY NONPOLYPOSIS COLORECTAL CANCER WITH MSH2 GENE PROMOTER METHYLATION

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Hereditary Nonpolyposis Colorectal Cancer Syndrome (HNPCC) is caused by germline mutation in one of the DNA mismatch repair (MMR) genes. Emerging data suggest that aberrant methylation of MMR genes can occur in the germline (soma-wide), causes cancer predisposition and with propensity for inheritance to offsprings. We have previously reported a HNPCC family with inheritance in three successive generations, of germline allele-specific and mosaic hypermethylation of the MSH2 gene promoter (Nat Genet 2006;38:1178-83). Similar phenomenon was observed in a second Chinese HNPCC family. To elucidate the underlying mechanism, linkage analysis was performed that revealed a 20 Mb chromosomal region linked with the occurrence of MSH2 methylation. This region encompasses the immediately upstream gene EPCAM (formerly known as TACSTD1). Interrogation of EST database showed a close association of the tissue-specific expression levels of EPCAM with presence of MSH2 methylation in affected individuals. A focus search using long range PCR and sequence analysis revealed a deletion of 22.8 kb (c.555+894_*14194del) extending from intron 5 of EPCAM to ~2.4 kb upstream of MSH2, leaving the MSH2 promoter intact (Nat Genet 2009;41:112-117). RT-PCR revealed presence of a fusion transcript, in which exon 5 of EPCAM was fused to exon 2 of MSH2, in blood leukocytes and colorectal mucosae of all family members carrying the deletions. The deletion and presence of fusion transcript co-segregated with the disease haplotype and MSH2 methylation. We next assessed the relative levels of fusion transcripts, wild type EPCAM and MSH2 respectively in blood leukocytes and colorectal mucosae, which then revealed a >100-fold higher level of fusion transcripts in colorectal mucosae compared to blood from EPCAM-deletion carriers, and that correlated with the levels of MSH2 methylation and wild-type EPCAM expression. A similar but smaller deletion of 4.9 kb encompassing the last two exons of EPCAM was detected in 4 Dutch HNPCC families, all associated with extended transcription of EPCAM into MSH2, and MSH2 methylation in both cancerous and/or normal colonic tissues. We concluded that deletion of the 3’ exons of EPCAM, including its transcriptional termination signals, leads to extended transcription into MSH2 and tissue-specific pattern of MSH2 methylation. Similar mechanisms may contribute to other types of genetic disease as well as somatic methylation in cancers driven by presence of frequent chromosomal aberrations.
COMPARING MODELS USED TO PREDICT MLH1, MSH2 AND MSH6 MUTATION CARRIERS: PROS, CONS AND PITFALLS.

William D Foulkes, Carly J Pouchet, Nora Wong, George Chong, Michael J Sheehan, Gretchen Schneider, Beth Rosen-Sheidley and Marc Tischkowitz

BACKGROUND: MMRpro, PREMM1,2 and MMRpredict are three models which have been developed to predict the probability that an individual carries a Lynch syndrome-causing mutation. Each model utilizes data from both personal and family histories of cancer. The purpose of this study was to determine each model’s ability to predict the probability of carrying a germline mismatch repair gene mutation in individuals with a family history of colorectal cancer, and to determine the clinical applicability of the models, in a cancer genetics clinic setting. METHODS: We obtained family pedigrees from 81 individuals who presented for HNPCC testing due to a personal and/or family history of cancer. Data from each pedigree were entered into MMRpredict, PREMM1,2 and MMRpro and were analyzed using SPSS. RESULTS: We found that MMRpredict, PREMM1,2 and MMRpro showed similar performances with areas under the ROC curve of 0.731, 0.765 and 0.732 respectively. PREMM1,2 showed the least dispersion of mutation probability estimates compared to the other two models with a p value of 0.205, compared to 0.034 for MMRpro and 0.001 for MMRpredict.

CONCLUSION: We found all three performed well in a cancer genetics setting, with PREMM1,2 giving slightly better estimates. However, there were some significant discrepancies between the models in cases where the proband had both endometrial and colorectal cancer. We will discuss situations where one model may be preferable over another, and will illustrate certain pitfalls of the various programs.
**LINKAGE TO CHROMOSOME 2Q32.2-Q35 IN FAMILIES WITH SERRATED NEOPLASIA**

Aedan Roberts, Derek Nancarrow, Daniel D. Buchanan, Mark Clendenning, David Duggan, Diane McKeone, Rhiannon Walters, Michael D. Walsh, Bruce W. Young, Jeremy R. Jass, Joanne P. Young

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**Background:** Causative genetic variants have to date been identified for only a small proportion of familial colorectal cancer (CRC). While conditions such as Familial Adenomatous Polyposis (FAP) and Lynch syndrome (Hereditary Nonpolyposis Colorectal Cancer, HNPCC) are caused by well defined genetic defects, the search for variants underlying the remainder of familial CRC is plagued by genetic heterogeneity. The recent identification of families with a heritable predisposition to malignancies arising through the serrated neoplasia pathway provides an opportunity to study a subset of familial CRC in which genetic heterogeneity may be greatly reduced.

**Methods:** A genome-wide linkage screen was performed on a large family displaying a dominantly inherited predisposition to serrated neoplasia genotyped using the Affymetrix GeneChip Human Mapping 10K Xba 142 Array, with parametric and nonparametric linkage analyses performed using Genehunter. Fine-mapping was undertaken in a further ten families using microsatellite markers spanning a 78 Mb region of interest on chromosome 2, and parametric linkage scores and haplotypes generated using SimWalk. LOD scores were also generated under the assumption of locus heterogeneity (HLOD). Lynch syndrome was excluded in all families using mismatch repair gene (MMR) immunohistochemistry and somatic BRAF mutation testing. Five candidate genes (CFLAR, CASP8, CASP10, FZD7 and BMPR2) were selected based on their biology and/or previous reports of association with colorectal cancer, and sequenced in family members.

**Results:** Genome-wide linkage analysis revealed a region on chromosome 2 with overlapping parametric (maximum LOD score 1.6) and nonparametric (maximum NPL 4.3) peaks. Fine-mapping further localised the region to 2q32.2-q35, with a total LOD score of 1.1 and HLOD of 2.8, with 7 of 11 families showing evidence of linkage. Haplotypes segregating with affected status were present in all 7 families. No segregating variants were found in the five primary candidate genes.

**Conclusions:** We have identified a 12 Mb locus on chromosome 2q with linkage to familial CRC arising through the serrated neoplasia pathway. Up to 60% of serrated neoplasia families may be linked to the 2q locus, but a causative variant is yet to be identified.
GERMLINE MUTYH MUTATIONS IN PATIENTS WITH HYPERPLASTIC POLYPOSIS SYNDROME

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Background: Mutations in the MUTYH gene are responsible for MUTYH-associated polyposis (MAP), a recessively inherited disorder predisposing carriers to polyposis (10-100 adenomas) and a high risk of colorectal carcinoma (CRC). Hyperplastic polyposis syndrome (HPS) is also characterised by multiple polyps throughout the colon, however, the polyps present demonstrate a serrated morphology in contrast to the adenomas of MAP. Recently, a series of 17 bi-allelic MUTYH mutation carriers reported that 8 of these individuals (47%) had at least one hyperplastic polyp, with 3 (18%) fulfilling the WHO criteria for HPS, raising the possibility that at least a proportion of HPS might be attributed to germline mutations in MUTYH. The aim of this study was to determine the frequency of the Y179C (formerly Y165C) and the G396D (formerly G382D) MUTYH mutations in patients with HPS.

Methods: The study comprised 126 patients with multiple serrated polyps from 120 families recruited from genetics clinics in Australasia (n=94) and North America (n=32) regardless of family history of polyps and cancer. Clinical and pathology data were extracted from histology reports, minimum polyp counts were derived from serial colonoscopy reports, where available, accounting for polyps removed at each procedure. Patients were recruited on the basis of either 20 or more hyperplastic/serrated polyps throughout the colon, or 5 hyperplastic/serrated polyps proximal to the sigmoid colon where the maximum size was 10 mm or greater in accordance with two of the WHO criteria for HPS. Patients with polyps demonstrating hamartomatous features were not included in the study. The Y179C and G396D MUTYH mutations were screened for using high resolution melt (HRM) assays, with the confirmation of aberrant melt profiles by direct sequencing.

Results: A single bi-allelic mutation carrier was identified, with the patient homozygous for Y179C mutation. The bi-allelic mutation carrier had at least 40 adenomas as well as more than 30 hyperplastic polyps throughout the colon. The remaining HPS patients in this series all had fewer than 25 adenomas. Three HPS patients were heterozygous for the G396D mutation.

Discussion: In this series of HPS patients a single bi-allelic MUTYH mutation carrier was identified displaying a phenotype suggestive of a mixed HPS and MAP lineage. Our data supports a limited role for mutations in the MUTYH gene in the aetiology of HPS.
LYNCH SYNDROME CANCER RISKS FOR MSH6 MUTATION CARRIERS


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Background
Germline mutations in *MSH6* account for 10-20% of colorectal cancer caused by DNA mismatch repair gene mutations. Cancer risks for mutation carriers are uncertain due to the few carriers studied.

Methods
We identified 113 individuals from five countries with *MSH6* gene mutations through family cancer clinics and population-based cancer registries. *MSH6* mutation status, sex, age, and cancer, polypectomy and hysterectomy histories were sought from 3,104 relatives of the individuals. Hazard ratios for cancer risks of carriers compared with those of the general population and age-specific cumulative risks for carriers were estimated using a modified segregation analysis.

Results
*MSH6* mutation carriers were estimated to have a 7-fold increased incidence of colorectal cancer (p<0.0001) independent of sex and age. Female carriers have a 22-fold increased incidence of endometrial cancer (p<0.0001) and 5-fold increased incidence of other cancers associated with Lynch syndrome (p<0.0001). For carriers, the estimated cumulative risks to age 70 and 80 years respectively were: for colorectal cancer 22% (95% CI 14% - 32%), and 44% (28%-62%) for males and 10% (5% - 17%) and 20% (11% - 35%) for females; for endometrial cancer, 26% (18% - 36%) and 44% (30% - 58%); and for other cancers associated with Lynch syndrome (small bowel, stomach, kidney, ureter, ovary, and brain) was 3% (6% - 19%) and 6% (1% - 25%) for males and 11% (6% - 19%) and 22% (12% - 38%) for females.

Conclusions
Male and female *MSH6* mutation carriers have substantially increased risk of colorectal cancer and the incidence does not appear to attenuate with age. Colorectal cancer risk for males is over twice that for females. Female carriers are at increased risk of endometrial and other Lynch syndrome cancers.
Programme

Wednesday 12th August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 3: Plantation Room

Chairperson: Stephen Fox
PATHOLOGICAL FEATURES OF ENDOMETRIAL CANCER IN PATIENTS WITH LYNCH SYNDROME: IMPLICATIONS FOR GENETIC TESTING.

José Palacios. Department of Pathology. Hospital Universitario Virgen del Rocío. Seville. Spain.

The incidence of endometrial cancer (EC) in women with Lynch Syndrome (LS) equals or exceeds that of colorectal cancer (CRC) and, in more than 50% of cases, these women present with a gynaecological cancer, EC or ovarian cancer (OC), as the first or sentinel malignancy. For individuals with documented MLH1 and MSH2 germline mutations, the lifetime risk of EC is estimated to be between 40% to 60%, and for women with MSH6 mutations as high as 70% by 70 years of age, compared to a lifetime risk of 1-2% in the general population. There is also an increased risk of OC with a lifetime risk of 8% to 12% compared to a lifetime risk of 1.45% in the general population. Both, EC and OC are diagnosed two decades earlier in LS patients than in the general population.

There are few studies analyzing pathological features in LS patients with documented germline mutations. Two of such studies including 73 EC (66 from MSH2 mutation carriers, 9 from MLH1 mutation carriers and 1 from a women with mutations in both genes) suggested that among these tumours there were a higher incidence (14% to 44%) of non-endometriod carcinomas (clear cells, serous and carcinosarcomas) than in age-matched control patients with sporadic EC (3% to 4%). Additional studies analysing EC with high levels of microsatellite instability (MSH-high) or loss of expression of mismatch repair (MMR) proteins in women with a familial history suggestive of LS have demonstrated that presumptive LS-associated EC were more frequently located in lower uterine segment, were more often poorly differentiated, and showed more frequently the presence of a Crhon-like lymphoid reaction, tumour infiltrating lymphocytes (TILs), lymphangioinvasive growth and deeper myometrial infiltration compared with sporadic EC. There is not current evidence suggesting that LS-associated EC have either a survival advantage or disadvantage when they are compared with sporadic cases.

Diagnosis of LS in patients with presenting with EC has important clinical implications for the individual and family members, since screening and prevention practices can decrease the likelihood of developing additional cancers. The true frequency of LS among newly diagnosed EC cases is difficult to asses from most studies because methodological differences among them. Three relatively large studies analyzing LS-germline mutations in unselected endometrial cancer patients found 1.8% to 2.1% mutations in MMR genes. However in series of EC patients younger of 50 years the incidence of germline mutations was between 4.9% to 9%. Age, familial history of LS-associated cancers and tumour pathological features, including the study of expression of MLH1,PMS2, MSH2 and MSH6 by immunohistochemistry (IHC), molecular testing of MSI and the analysis of promoter hypermethylation of MLH1 (to identify MSH-high sporadic EC with loss of expression of MLH1/MSH2) can help in select women with EC for LS genetic testing. In patients with EC, IHC is sensitive and cost-effective to detect MSI. In addition it can guide genetic testing to specific genes and identify those EC cases without or low MSI due to MSH6 germline mutations.

Summarizing, a combination of family and personal history and tumour testing provides an efficient basis for diagnosis of LS in women with EC.
ADENOMAS IN LYNCH SYNDROME: DIAGNOSTICALLY USEFUL?

Michael Walsh, Daniel Buchanan, Sven Arnold, Mark Clendenning, Rhiannon Walters, John Hopper, Mark Jenkins, Jeremy Jass, and Joanne Young

Queensland Institute of Medical Research, Brisbane, AUSTRALIA, University of Melbourne, Carlton, AUSTRALIA, and St Mark’s Hospital, Harrow, UK

Background: Debate continues as to the usefulness of testing adenomas for loss of mismatch repair (MMR) proteins to identify individuals with suspected Lynch syndrome. Several studies have suggested that it is only worthwhile testing large, proximal adenomas exhibiting high grade dysplasia while others report disappointing “hit rates” even from known mutation carriers. Screening of unselected early-onset adenomas for MMR deficiency has yielded disappointing results.

Design: We tested 88 adenomas from 49 proven mutation carriers (23 female and 26 male) from 37 Lynch syndrome families (12 MLH1, 21 MSH2, 3 MSH6 and 1 PMS2) enrolled in the Australasian Colorectal Cancer Family Study which is part of the Colon Collaborative Family Registry (C-CFR). The average age of diagnosis of first polyp studied was 49.1 ± 9.6 years (ranging from 30.3 to 72.0 years).

All polyps were tested by immunohistochemistry (IHC) for the four MMR proteins MLH1, MSH2, MSH6 and PMS2. In addition, microsatellite instability testing using a panel of 10 markers (incorporating the standard NCI panel) was performed on 69 adenomas. All polyps were subjected to a standard review by one specialist pathologist (JJ). The majority of adenomas included in the present study were tubular adenomas (n = 61), with 22 tubulovillous adenomas, 1 villous adenoma, and 4 traditional serrated adenomas.

Results: Overall, abnormal immunohistochemical results (loss of expression) were noted for 73/88 (82.9%) of the adenomas from MMR gene mutation carriers. In all instances, loss of expression was consistent with the underlying germline mutation. MSI testing was concordant with immunohistochemistry in 66/69 (95.6%) cases. There was no statistical difference in patient ages at the time of polypectomy between MMR deficient and intact adenomas (49.6 ± 9.5 yrs vs. 49.8 ± 11.6 yrs). 15/88 (17.1%) polyps demonstrated normal MMR IHC. Six of these polyps came from four individuals who had other polyps which demonstrated abnormal IHC), whilst the remaining nine adenomas came from nine individuals for whom other adenomas had not been tested. Of these 9 cases, however, three had been diagnosed with Lynch syndrome-spectrum tumours which showed appropriate loss of MMR protein(s).

Conclusions: Diagnostic testing of adenomas from patients from Lynch syndrome families is a useful alternative in cases where cancers are unavailable. The overwhelming majority (82.9%) of adenomas from carriers show appropriate loss of MMR proteins and a high level of concordant microsatellite instability. We were unable to demonstrate an association between adenoma grade, size or site and MMR deficiency.
INTRA-FAMILIAL CONCORDANCE IN BREAST CANCER PATHOLOGY AS AN INDICATOR OF GENOTYPE IN FAMILIAL BREAST CANCER.

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The heterogeneous nature of multiple case breast cancer families that do not carry mutations in \textit{BRCA1} or \textit{BRCA2} (non-\textit{BRCA1/2} families), poses a challenge to identification of further breast cancer susceptibility genes. Germ-line mutations in certain genes, including \textit{BRCA1} are known to be associated with certain histopathologic features of breast cancer. The aim of this study was to determine whether the degree of intra-familial concordance in breast cancer histopathology could assist identification of non-\textit{BRCA1/2} families with consistent genotypic features.

Systematic histopathology review was completed for invasive breast cancers from 84 individuals belonging to 30 kConFab families. This included families carrying pathogenic mutations or unclassified sequence variants in \textit{BRCA1} (n=9) and \textit{BRCA2} (n=10) as well as non-\textit{BRCA1/2} families (n=11). There were between one and six breast cancers examined per family.

An unsupervised hierarchical clustering analysis approach based on histopathologic features and age at diagnosis, sub-divided breast cancers into three distinct cluster groups. In general, cluster 1 were uniformly high grade cancers that tended to show \textit{BRCA1}-mutation associated features, cluster 2 cases were also predominantly high grade with less pronounced \textit{BRCA1}-associated features and cluster 3 breast cancers were low (38%) or intermediate grade (51%). According to this sub-classification, breast cancers in \textit{BRCA1} mutation carriers belonged predominantly to cluster 1, while \textit{BRCA2} mutation carrier families showed mostly mixed cluster pathology.

By analogy, non-\textit{BRCA1/2} families were categorised according to whether they showed cluster concordant or cluster mixed breast cancer pathology. Genome-wide linkage data were then separately analysed for cluster 3 concordant (n=5) and cluster mixed non-\textit{BRCA1/2} families (n=4). This demonstrated a range of distinct, non-overlapping linkage peaks that did not correspond to the location of known breast cancer susceptibility genes.

The degree of intra-familial concordance in breast cancer histopathology may assist location of novel breast cancer susceptibility genes by focussing genetic analyses on phenotypically distinct subgroups of non-\textit{BRCA1/2} families.
Programme

Wednesday 12th August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 4: Plantation Room

Chairperson: David Bowtell

“Familial Cancer 2009: Research and Practice”
STROMAL-EPITHELIAL INTERACTIONS IN OVARIAN CANCER: IMPLICATIONS FOR BIOLOGY AND TREATMENT

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The important role of tumor stroma in determining the clinicopathologic properties of many malignancies has recently emerged. The precise mechanisms underlying signaling between tumor-associated stroma and adjacent ovarian epithelial tumor cells remain unclear. To determine this we analyzed gene expression profiles of purified populations of fibroblasts microdissected from 10 normal ovaries and 51 high grade papillary serous tumors. Unsupervised hierarchical clustering utilizing 9,741 filtered probe sets clearly distinguished tumor-associated from normal stroma. Class comparison analysis identified 2,703 differentially expressed probe sets in tumor-associated versus normal stroma ($p<0.001$). To validate the array results, multiple differentially expressed genes were selected for real-time PCR analysis across all of the samples. Eighty-nine percent were found to be significantly up-regulated in tumor-associated stroma. Analysis of the differentially expressed gene list identified multiple TGF-β-regulated genes activated in the tumor-associated stromal isolates.

This increase in expression of TGF-β-regulated stromal genes occurs in parallel with gene alterations within the tumor epithelium which blocks the growth inhibitory effects of TGF-β. Many of these stromal genes would be predicted to stimulate tumor cell growth. Stromal over-expression of one of these TGF-β-regulated proteins, CTGF, was confirmed by immunohistochemistry. Biological studies of CTGF overexpression in the ovarian cancer cell line A547 revealed increased activity of processes involved in tumor progression. Compared to empty vector controls, CTGF-transfected clones demonstrated higher proliferation rates (32.0 ± 5.5 versus 42.8 ± 14.0 hrs), anchorage-independent growth (20.7 ± 11.6 versus 5.6 ± 1.8 colonies), migration (71.8 ± 38.7 versus 6.2 ± 3.5 cells) and invasion (212.4 ± 7.2 versus 1.9 ± 1.5 cells). Protein such as CTGF, TGF-β-regulated proteins or TGF-β itself may make convenient and important therapeutic targets.

As a second level bioinfomatic analysis we filtered our expression data for secreted proteins, which would be expected to impact on the biology the neighboring malignant epithelial cells. Identifying those genes (probe sets), which are likely to be secreted expressed on the membrane, enabled us to create and seceterome array. Overlaying this onto the differential gene expression data identified 44 secreted genes, which are differentially expressed within the stroma. Interestingly, several genes are known to be associated with the maintenance of tumor stem cells. Validation studies identified mRNA of multiple stem cell-related genes (including multiple collagen genes, BGN, sFRP4 and sFRP2) expressed at significantly higher levels in the cancer-associated stroma versus the tumor epithelium and the normal ovarian stroma ($p<0.001$). Further characterization demonstrated that several of these are statistically significantly associated with poor patient survival and resistance to chemotherapy. Differential gene expression within the stroma may be critical for the establishment of a stem cell niche for ovarian cancer.
ARE THERE ANY MORE OVARIAN TUMOUR SUPPRESSOR GENES? A NEW PERSPECTIVE USING ULTRA HIGH RESOLUTION COPY NUMBER AND LOSS OF HETEROZYGOSITY ANALYSIS

Kyli e L. Gorringe\textsuperscript{1,2}, Manasa Ramakrishna\textsuperscript{1,2}, Louise H. Williams\textsuperscript{1}, Anita Sridhar\textsuperscript{1}, Samantha E. Boyle\textsuperscript{1}, Jennifer L. Bearfoot\textsuperscript{1,2}, Jason Li\textsuperscript{3}, Michael S. Anglesio\textsuperscript{4}, Ian G Campbell\textsuperscript{1,2}

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Ovarian cancer is characterized by complex somatic genetic alterations, including copy number loss and copy number-neutral loss of heterozygosity (LOH). These alterations are assumed to represent the “second hit” of the underlying tumour suppressor gene (TSG), however, relative to the number of LOH hotspots reported, few ovarian TSGs have been identified. We conducted a high resolution LOH analysis using SNP arrays (500K and SNP6.0) of 106 primary ovarian tumours of various histological subtypes together with matching normal DNA. LOH was detected in at least 35% of samples on chromosomes 17, 19p, 22q, Xp, 13q, 8p, 6q, 4q, 5q, 1p, 16q and 9q with a median minimal region of overlap of only 300 kb. Subtype-specific differences in LOH frequency were noted, particularly for mucinous cases. We also identified 192 somatic homozygous deletions (HDs). Recurrent HDs targeted known TSGs such as \textit{CDKN2A} (8 samples), \textit{RB1} (5 samples), and \textit{PTEN} (3 samples). Additional recurrent HDs targeted 16 candidate TSGs near minimal regions of LOH on chromosomes 17, 13, 8p, 5q, and X. Given the importance of HDs in inactivating known genes, these candidates are highly likely to be ovarian TSGs. Our data suggest that the poor success of previous LOH studies was due to the inability of previous technology to resolve complex genomic alterations and distinguish true LOH from allelic imbalance. This study shows that recurrent regions of LOH and HD frequently align with known TSGs suggesting that LOH analysis remains a valid approach to discovering new candidates.
SOMATIC GENOMIC ALTERATIONS AND EXPRESSION PROFILING LINK STAT3 ONCOGENIC PATHWAYS TO OVARIAN CLEAR CELL CARCINOMA

Michael S Anglesio, Joshy George, Kylie L Gorringe, Manasa Ramakrishna, Sian Fereday, Australian Ovarian Cancer Study Group, Ian G Campbell, Anna deFazio, C Blake Gilks, David DL Bowtell

Ovarian Clear Cell Carcinoma (OCCC) represents ~6% of ovarian epithelial malignancies in western countries and up to 20% in Japanese populations. Although they are often diagnosed at an early stage, OCCC tend to be chemoresistant and women with OCCC have a shorter median survival than their stage-matched serous counterparts. Compared with serous cancers, there has been relatively little investigation of the molecular features of OCCC. Gene expression studies have suggested that OCCC have an expression profile divergent from serous carcinomas. A small number of tumour chromosome studies of DNA copy number, primarily traditional CGH, have been performed, however, high-resolution profiling of copy number aberrations remain largely under-characterised. This study aims to integrate data from somatic DNA copy number changes (CNC) with gene expression profiling using OCCCs obtained from the Australian Ovarian Cancer Study (AOCS).

Supporting previous studies, unsupervised clustering of our cohort based on expression data alone clearly demonstrated a unique molecular profile for OCCC compared to other malignant ovarian cancers. Somatic CNC changes in OCCC were less frequent overall than in high-grade serous ovarian carcinomas, with the most frequently affected regions being gains on 8q, 20q and 3q, and losses on Chr 9. To focus on “driver events” for the clear cell phenotype we employed a simple peak finding strategy to identify minimal regions of gain or loss in copy number frequency data, and then focused on differentially expressed genes within these regions. Using CNC and expression data together we identified a number of genes that were both amplified in a subset of tumours, and up-regulated across our cohort, whose function suggests upstream activation of the STAT3 oncogene pathway including: MET, ERBB2, ERBB3, PRLR, the src family kinase LYN, and STAT3 itself. In addition, the overall gene expression profile showed a significant enrichment of STAT3 related ontologies including the canonical JAK/STAT pathway, inflammatory cytokine pathways, and Acute Phase Response.

Two TMA validation panels comprising > 150 OCCC tumours are currently being evaluated by immunohistochemistry for activation of STAT3 (phospho-STAT3) and expression of upstream (LYN), and downstream (HIF1α) elements of the STAT3 pathway. We have also examined gene expression and CNC profiles of OCCC derived cell lines to identify representative model systems for functional analysis of the STAT3 pathway in OCCC. These cell lines will be assessed for changes in growth or sensitivity to traditional chemotherapeutic agents following gene knockdown using RNAi or specific inhibitors.
GENOME-WIDE LOH AND HAPLOTYPic ANALySES OF BRCAX TUMOURS IDENTIFY CANDIDATE REGIONS FOR HIGH RISK BREAST CANCER SUSCEPTIBILITY.

Ella Thompson, Ian Campbell
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The ability to identify disease-causing mutations in high risk breast cancer families has broad implications for those affected, in terms of risk assessment and management as well as treatment. With as much as 75% of the excess familial risk of breast cancer yet to be attributed to genetic variants, there is much progress to be made in our understanding of the disease. We have adopted a novel, genome-wide strategy for the identification of high risk breast cancer susceptibility (‘BRCAx’) genes using single nucleotide polymorphism (SNP) arrays to assess somatic allele loss in hereditary breast tumours and exploit the likelihood that BRCAx genes function as classical tumour suppressor genes (as is the case for BRCA1, BRCA2 and other familial cancer genes). We hypothesise that the analysis of overlapping regions of loss of heterozygosity (LOH) in multiple related tumours should define a candidate region in which a BRCAx gene resides. Furthermore, haplotype analysis will refine this candidate region since the mutant allele will be retained in all related tumours.

Frozen and formalin-fixed, paraffin-embedded breast tumours were collected from kConFab BRCAx families and assessed for LOH and haplotype sharing using Mapping 250K arrays. Between one and two tumours and up to five additional germline samples were analysed in each of six BRCAx families. LOH analysis of a single tumour in most families refined the candidate regions to a genomic area encompassing 440 Mb per family on average (range 176–780 Mb). Subsequent haplotype sharing analysis of tumour and germline samples further refined the candidate regions to just 69 Mb per family on average (range 31–136 Mb). These minimal candidate regions contain an average of 472 candidate genes (range 176–1,001) and 17 snoRNAs/miRNAs per family. The power of an intra-familial, tumour-based strategy was clearly demonstrated by the characterisation of candidate regions using data obtained from very limited numbers of tumour and germline samples.

The number of genes contained within these candidate regions are too numerous to assess using conventional capillary sequencing on a gene-by-gene basis. However, the recent development of massively parallel (or ‘next generation’) sequencing (NGS) coupled with target enrichment protocols enables the simultaneous sequencing of hundreds or thousands of candidate genes. Therefore, we are preparing to use targeted NGS in combination with familial LOH analysis to identify pathogenic germline mutations in novel BRCAx genes.
Programme

Thursday 13th August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 5: Plantation Room

Chairperson: Margaret Gleeson
AS GENETICS MOVES INTO MAINSTREAM MEDICINE: CHALLENGES FACING GENETIC COUNSELORS

Barbara Biesecker, M.S., CGC National Human Genome Research Institute, NIH

Genetic counselors all across the globe provide a specialty service that contrasts with primary care medicine. We have specific expertise on assessing familial risks, personal decision-making, health education, psychological counseling and use of support/advocacy resources related to genetics. With a movement toward personalized or genomics medicine and the integration of genetics into primary care, our services will evolve. Inherent to the process of moving to a more primary care model, clients will be inadequately served. While it’s tempting to predict the lapses of histories untaken, tests not ordered and counseling issues unaddressed, it’s important to reframe this transition as a time of opportunities. Primary care providers will need training in taking efficient and effective histories. They will seek guidance about which cases to manage in their office and which may need to be referred out. Further, there will be a need for resources to help with ordering and interpreting genetic test results. All of these needs suggest opportunities for genetic counselors to educate primary care providers, develop on-line modules, provide consultation services, and develop tools that can be used in the clinic. It behooves us to stay abreast of the literature on interpreting results of new genetic sequencing tests. Perhaps most important to this transition, is the opportunity to position ourselves as resources for more complex cases. We need not spend the next decade handing off our specialty services to primary care providers without positioning ourselves as even more essential experts in the care of genetics clients. To make this happen, we must articulate our expertise and demonstrate its application, develop resources that can be exported, study the essence of what we do that makes a difference in the quality of our clients’ lives and establish ourselves as the professionals to turn to when the genomics results on a client are beyond what the primary care providers can address. We need to become leaders in this transition and position ourselves in future health delivery models of genetics service provision. Examples of ways to accomplish this transition to favor the credibility and expertise of genetic counselors will be discussed.
DELIVERING GENETIC COUNSELLING VIA TELEHEALTH: AN EXPLORATION OF PRACTITIONERS' AND PATIENTS' EXPERIENCE OF A VIRTUAL CONSULTATION

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Telehealth, or videoconferencing, is an evolving field in cancer genetics. We undertook two separate qualitative studies to explore clinicians’ and patients’ perspectives and experiences of telehealth genetic counselling for hereditary breast/ovarian cancer (HBOC). As part of Study 1, semi-structured interviews were conducted with a sample of twelve women who had received telehealth within the past 12 months for HBOC and as part Study 2, fifteen interviews were conducted with genetic clinicians and genetic counsellors practicing telehealth in New South Wales and the ACT. The interviews explored experience, satisfaction, aims of the service, advantages and disadvantages of the technology, practitioner roles within the consultation and the perceived quality of the interaction between the patient and their genetic professionals. The interviews were recorded, transcribed verbatim and analysed for recurrent themes. RESULTS: Study 1: Overall patients were highly satisfied with telehealth and reported increased convenience and reduced travel and associated costs. The majority of women described feeling a high degree of social presence of, or rapport with, the off-site genetic clinician. One patient, who underwent rapid testing following a recent breast cancer diagnosis and underwent complex decision-making, felt telehealth had been inadequate for psychosocial support and education. Study 2: Practitioners reported high levels of satisfaction with telegenetics and increased staff efficiency and accessibility to outreach clinics. Practitioners reported that during telehealth, the genetic clinician presented as the consulting specialist, delivering medical information and screening advice and depended more on the genetic counsellor to assess non-verbal behaviour and subtle emotional cues from the client. Consultations were described by practitioners as being more formal, and possibly less open to emotional expression than face-to-face consultations. Interactions on-screen were moderated by the physical positioning of the genetic counsellor and client. When the counsellor was positioned “off screen”, a medically modelled dyadic interaction occurred and nonverbal cues between the counsellor, the geneticist and the client were obscured. When positioned “on screen”, counsellors reported they offered a higher level of psychosocial support before, during and after the telehealth session. CONCLUSION: Study 1: Patients attending for HBOV genetic counselling were highly satisfied with the technology and the interaction. The technology was found to be efficient and offer sufficient resolution to attend to most patients’ psychosocial needs; however care should be taken with patients with more complex psychosocial needs. Study 2: Practitioners were highly satisfied with telegenetics but acknowledged the trade off involved in the geneticist not being physically present. Findings highlight the benefits of co-facilitation between geneticists and genetic counsellor when each has clearly defined complementary roles.
TELEPHONE CLINICS: AN ALTERNATIVE METHOD OF DELIVERING CANCER GENETIC COUNSELLING SERVICES

Linda Cicciarelli, Mary-Anne Young, Chris Michael-Lovatt, Lucinda Hossack, Gillian Mitchell. Peter MacCallum Familial Cancer Centre, Melbourne, VIC

Greater awareness by health professionals and the public of the role of genetic factors in the aetiology of cancer has resulted in an ever increasing number of referrals to Familial Cancer Clinics (FCC) for genetic risk assessment and genetic counselling.

In order to address the ongoing increase in demand for FCC services, the FCC at Peter MacCallum Cancer Centre have implemented a telephone clinic based on the model originally developed at the Royal Marsden NHS Foundation Trust (RMH) Cancer Genetics Clinic. This model has been shown to provide an alternative way of delivering genetic counselling services that is efficient for patients with a focus on equity and access. As well it is cost effective and allows optimal utilization of current resources.

Telephone appointments are conducted by the genetic counsellor for patients assessed at low risk, moderate risk, some high risk patients or patients who have specific issues that can be managed without the presence of a medical specialist. This enables the standard combination of genetic counsellor and medical specialist to its best advantage in seeing patients face to face who have a potentially high risk of cancer and may require a more complex discussion about cancer risk management. A 12 week evaluation following implementation of this model showed 72 patients were triaged into telephone clinic appointments. Of these patients:

- 8% had family history collected as did not return their family history questionnaire
- 19% were assessed at low risk
- 56% were assessed at moderate risk
- 7% were assessed at high risk and did not require a medical specialist
- 10% did not respond for their telephone appointment (failed to attend)

One consideration arising from conducting telephone appointments is the issue of heightened levels of patient anxiety which can be difficult to assess without the visual cues of a face to face appointment. This required a different use of practical counselling strategies to provide these patients with support and reassurance following their assessment of risk.

Following their telephone appointment, 81% of patients required no additional follow-up and were discharged from the clinic indicating a more efficient and effective way to manage patients. This approach has enabled improved use of current resources, a reduction in waiting times for clinic appointments and staff expressing their skills are better utilised for high risk patients. Further evaluation will involve patient and staff feedback to help facilitate the ongoing development of this type of service delivery.

This model has become an integral part of our service and could ultimately be adopted by other genetic counselling services as a proven method of meeting an ever increasing demand and help provide a flexible patient-centred approach to the delivery of genetic services.
UPDATING FAMILIAL CANCER RESOURCES FOR CONSUMERS: AN EVIDENCE BASED APPROACH

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Given the rapid progress in the field of cancer genetics, consumer focused education materials addressing topics relevant to families affected by hereditary cancer require regular evaluation and updating. As this is costly, evidence is needed of the demand for this information, past and future potential usage and format preferences. Funding was received to support this need from The Cancer Council NSW as part of a larger Strategic Research Partnership Grant.

An audit and evaluation of key familial cancer resources was undertaken. The audit used the CGE electronic database which tracks resource dissemination in NSW and interstate. An evaluation was undertaken with the NSW Familial Cancer Services and outreach genetic counselling services, as well as other interstate users. 28/42 completed the evaluation which sought to determine how the resource were used, preferred format, and desired edits. Findings for all the resources were similar with preference for format being print (61% - 69%) with some wanting both print and electronic formats (31%-33%). Generally, for those who provided the material to clients (67% - 83%), half used the material in consultations and half sent it home with the patient. The majority requested the information to be updated.

Since the audit and evaluation, two of the identified key resources addressing preventative surgery to manage cancer risk have undergone expert peer review, including consultation with original researchers and consumer testing; Information For Women Considering Preventative Mastectomy Because of a Strong Family History Of Breast Cancer and Risk Management Options For Women at Increased Risk Of Developing Ovarian Cancer. These materials are available in print and electronic format.

The development of two decision aids on genetic testing for familial cancer risk is also near completion.
Programme

Thursday 13th August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 6: Plantation Room

Chairperson: Amanda Spurdle
GENOME WIDE ASSOCIATION STUDIES TO IDENTIFY GENETIC RISK FACTORS FOR BREAST CANCER AND GENETIC MODIFIERS OF BREAST AND OVARIAN CANCER RISK IN BRCA1 and BRCA2 MUTATION CARRIERS

Fergus J. Couch on behalf of CIMBA and TNBCC investigators

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA.

Genome wide association studies (GWAS) by a number of consortia, including the Breast Cancer Association Consortium (BCAC), have been very effective in identifying genetic risk factors for breast cancer. Most of the risk factors identified to date are associated with risk of estrogen receptor positive, but not with estrogen receptor negative breast cancer, suggesting that specific subtypes of breast cancer may be associated with specific risk factors.

Based on these findings, we have recently formed the Triple Negative Breast Cancer Consortium (TNBCC) with the goal of identifying genetic risk factors for this subtype of breast cancer. Triple negative breast cancer accounts for approximately 15% of all breast tumors and is defined by low expression or absence of ER, PR and Her2. These tumors tend to exhibit large size at presentation, higher grade, and high proliferation rate and have a corresponding poor clinical outcome. We have initiated a two-stage GWAS using DNA from 5,400 triple negative breast cancer cases and matched unaffected controls provided by 44 TNBCC groups. The approach to this study will be outlined. Risk factors identified through this study will provide insight into the etiology of this subtype of breast cancer and may prove useful for targeting of therapeutic and preventive agents.

Several of the known genetic risk factors for breast cancer have also been evaluated as genetic modifiers of breast cancer risk in BRCA1 and BRCA2 mutation carriers by the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Most of these have shown specific associations with breast cancer in BRCA2 mutation carriers but not in BRCA1 mutation carriers. This is consistent with the knowledge that BRCA1 tumors display predominantly ER negative and triple negative pathology. To identify genetic modifiers of breast cancer risk in BRCA1 and BRCA2 mutations carriers two GWAS studies have been initiated. For the BRCA1 GWAS a total of 11,000 DNA samples from BRCA1 mutation carriers have been collected. Stage 1 of the BRCA1 GWAS has been completed and candidate modifiers are being re-evaluated in Stage 2. Details of the BRCA1 GWAS will be provided.

Because BRCA1 mutation carriers also develop ovarian cancer at high frequency, the BRCA1 GWAS has been extended to consider ovarian cancer as an endpoint. A Stage 1 study of 850 BRCA1 carriers affected with ovarian cancer is now complete. The details of this study will be provided.

At the conclusion of the BRCA1 GWAS we expect to identify risk modifiers that may prove useful for improved risk assessment for BRCA1 mutation carriers, while also providing utility for etiology, therapeutics and prevention.
FAMILIAL BREAST CANCER IDENTIFIES DISTINCT PROFILES DEFINED BY MUTATION STATUS
James M. Flanagan¹, Sibylle Kugler², Nic Waddell², Cameron Johnstone², Anna Marsh², Stephen Henderson¹, Peter Simpson³, Leonard da Silva³, kConFab Investigators⁴, Sunil Lakhani³, Chris Boshoff³ and Georgia Chenevix-Trench²

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3. Department of Pathology, University of Queensland, Brisbane, Queensland
4. Peter MacCallum Cancer Centre, East Melbourne, Victoria

The occurrence of promoter CpG island methylation is a well known phenomenon in many cancers and it is now well understood that epigenetic alterations occur frequently in sporadic breast cancer. However, little is known about its role in familial breast cancer. To address this issue, we performed genome-wide DNA methylation profiling on 33 frozen familial breast cancer specimens, including *BRCA*1 (n=11), *BRCA*2 (n=8) and non-*BRCA*1/2 (n=14) tumours to identify specific methylation patterns in breast cancer mutation subgroups and/or intrinsic, ‘Sorlie’ subtypes. We used methylated DNA immunoprecipitation (meDIP) on Affymetrix human promoter chips to determine methylation profiles across 25,500 distinct transcripts.

Using a support vector machine learning algorithm, we showed that genome-wide methylation profiles predict the tumour mutation status with 64-81% accuracy, but do not accurately predict the intrinsic subtypes defined by gene expression (46% and 57% for luminal A and basal-like tumours, respectively). Interestingly, using unsupervised hierarchical clustering we could identify a distinct subgroup of non-*BRCA*1/2 tumours defined by methylation profiles. These findings were further verified by methylation analysis of promoter regions of selected genes with pyrosequencing and EpiTyper technology. We also showed that genes previously reported as hypermethylated in sporadic breast cancer are also methylated in familial tumours. Finally, integrated analysis with gene expression and SNP CGH profiling previously performed on the same samples revealed frequent hypermethylation of genes that also displayed loss of heterozygosity (LOH) compared to the same gene in diploid tumours. We also observed frequent hypermethylation of genes that show copy number gains compared to diploid tumours, providing a potential mechanism for expression dosage compensation. Taken together these results suggest distinct methylation profiles for familial breast cancers are defined by the mutation status, and differ from the intrinsic subtypes.
EVIDENCE FOR SMAD3 AS A MODIFIER OF BREAST CANCER RISK IN BRCA2 MUTATION CARRIERS BUT NOT BRCA1 CARRIERS

Logan C Walker¹, Zachary Fredericksen², Robert Tarrell², Vernon S. Pankratz², Xianshu Wang², Jonathan Beesley¹, Xiaqing Chen¹, kConFab³, GEMO⁴, GHC-HBOC⁴, DNA-HEBON⁴, ModSQuaD⁴, EMBRACE⁴, UPPEN⁴, MUV⁴, FCCC⁴, Georgetown⁴, HEBCS⁴, Helsinki⁴, ILUH⁴, INHERIT⁴, MAGIC⁴, MAYO⁴, OUH⁴, Poland⁴, PISA⁴, SWE-BRCA⁴, Antonis C. Antoniou⁵, Georgia Chenevix-Trench¹, Fergus J. Couch², Amanda B Spurdle¹*

¹Queensland Institute of Medical Research, Australia; ²Mayo Clinic, Rochester, Minnesota, USA; ³Peter MacCallum Cancer Centre, Melbourne, Australia; ⁴Collaborators participating in the CIMBA consortium, ⁵University of Cambridge, UK

Germline mutations in BRCA1 or BRCA2 confer an increased lifetime risk of breast or ovarian cancer but variable penetrance suggests that these risks are modified in part by commonly inherited genetic variation. Current attempts to identify genetic modifiers of BRCA1 and BRCA2 associated risk have focused on a candidate gene-based approach or the development of large genome wide association studies. However, both methods have notable limitations. In a previous study, we used a novel combinatorial approach for analyzing gene expression differences in irradiated lymphoblastoid cell lines (LCLs) from BRCA1 or BRCA2 mutation carriers to prioritize candidate modifier genes for single nucleotide polymorphism association studies. The advantage of this strategy over other candidate gene-based studies is the potential to identify candidate genes that interact with exogenous risk factors to cause or modify cancer, without detailed a priori knowledge of the molecular pathways involved. As a result, 20 irradiation responsive genes were identified as high priority candidate BRCA1 and/or BRCA2 modifier genes. In the present study, we genotyped 33 tagged single nucleotide polymorphisms (SNPs) from these 20 candidate genes on 5428 BRCA1 and 2930 BRCA2 female mutation carriers from eight groups to determine whether the SNPs modified breast cancer risk in BRCA1 and BRCA2 mutation carriers. None of the SNPs analysed were significantly associated with risk in BRCA1 mutation carriers. However, the minor alleles of two SNPs (rs7166081 and rs3825977) located at and near the SMAD3 gene at chromosome 15q22.33 were each associated with increased breast cancer risk in BRCA2 mutation carriers (per-allele hazard ratio [HR] = 1.23, 95% CI: 1.06 – 1.43, \( P_{\text{trend}} = 0.006 \) and HR = 1.21, 95% CI: 1.04 – 1.41, \( P_{\text{trend}} = 0.013 \)). SMAD3 is a critical factor in the intracellular signalling of TGFβ, which is an inhibitor of epithelial cell proliferation. SMAD3 is also known to form a complex with BRCA2. Preliminary analysis of SMAD3 mRNA levels in untreated LCLs from BRCA2 mutation carriers has revealed that in relation to full-length SMAD3 mRNA the delta3-isoform may be expressed at lower levels in heterozygous variant carriers compared to homozygous wild-type individuals. Furthermore, the extent to which full-length SMAD3 expression is decreased in the same LCLs as a result of irradiation treatment appears also to be associated with genotype. Thus, preliminary results suggest that sequence changes at the SMAD3 locus may be associated with altered expression of SMAD3 and breast cancer risk in BRCA2 mutation carriers.
FROM TUMOUR-TYPE TO GENOTYPE: A NEW PARADIGM FOR BREAST AND COLORECTAL CANCER GENETICS SERVICES THAT WILL SAVE LIVES

John L. Hopper, Melissa C. Southey and Mark A. Jenkins for the Australian Breast Cancer Family Registry and the Australasian Colorectal Cancer Family Registry

Traditionally, entry to cancer genetic services for possible genetic testing has been based on family history. For breast cancer, data from the UK (Antoniou et al., 2007) and kConFab (representing experiences in Australia and New Zealand) show that less than 20% of clinic-based families tested have been found to be segregating high-risk mutations in known susceptibility genes. For colorectal cancer, about one-half of families meeting the Amsterdam II criteria are being found to segregate high-risk mutations in the mismatch repair genes. Families attending these clinics are typically offered non-directional genetic counselling and are not necessarily followed-up with appropriate clinical care and management.

On the other hand, Australian population-based studies have shown that, by conducting molecular and/or morphological studies of tumours of early-onset cases, mutation carrying cases can be identified with much higher sensitivity and specificity. Furthermore, identification of carriers among early-onset cases has the potential for these people to receive better treatment, and for their risk of death from this or subsequent cancers reduced. Their genetically at risk relatives can be identified and offered appropriate screening, again with the potential to reduce death from cancer. All this can be supervised from a disease-specific clinical setting.

A change in emphasis by cancer genetics services to focus more on early-onset cases with appropriate tumour-based triaging for mutation testing could lead to a major reduction in deaths caused by hereditary breast and colorectal cancer.

We will present data to support the above, discuss how to overcome barriers to the implementation of this new paradigm, and present ideas on how this can be achieved in Australia.
Programme

Thursday 13th August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 7: Pavilion Room

Chairperson: Gillian Mitchell
GERMLINE STUDIES IN SARCOMAS

DM Thomas, M Ballinger, S Young & Australian Sarcoma Study Group (ASKS) Research Department & DHMO, Peter MacCallum Cancer Centre.

Sarcomas are a group of diverse, rare cancers which predominantly affect a younger population. They comprise 20% of childhood cancer, 10% of cancer in young adults and 1-3% of cancer overall. Age of onset is a powerful predictor of inherited cancer risk, and sarcomas have traditionally yielded a rich trove of familial cancer research. Moreover, the incidence of second malignancy in sarcoma patients approaches 20%.

However this research has been overwhelmingly focused on paediatric sarcomas, a distinct subset totalling 10% of all sarcomas. There has been traditionally little research in adult-onset sarcomas. We have undertaken preliminary studies examining the role of polymorphisms in the MDM2 gene in sarcomas and in germline material.

The somatic genetics of sarcomas may distort the interpretation of SNP309 allele frequencies in this population, because of the common high level amplification in tumours reported to carry the oncogenic G-allele. In addition, we have identified the unexpected finding of Met variants at high frequency in the germline of patients with myxoid liposarcoma, together with loss of miRNA-mediated Met repression in these tumours.

Together, these data suggest that a systematic analysis of both the somatic and germline genetics of adult-onset sarcomas will continue to provide insights into the biology of cancer.
CANCER TREATMENT AND PREVENTION BY REVERSAL OF THE GLYCOLYTIC PHENOTYPE IN A MODEL OF LI-FRAUMENI SYNDROME

Anneke C. Blackburn1, Melissa Rooke1, Ramon C. Sun1, Mitali Fadia2, Jane E. Dahlstrom2, Philip G. Board1
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Over 90% of all tumours show an increase in glycolysis, glucose uptake and lactate production known as the “glycolytic phenotype”. Dichloroacetate (DCA), a drug used for lactic acidosis treatment, has recently been described as a novel and relatively non-toxic anti-cancer agent which can reverse the glycolytic phenotype. We have previously shown the ability of DCA to inhibit the growth of metastases in vivo in a rat model of metastatic breast cancer (1). We are now examining the effectiveness of DCA in breast cancer treatment and prevention in the spontaneous BALB/c-Trp53+/- mouse mammary tumour model.

In vitro, growth of the V14 cell line (derived from a BALB/c-Trp53+/- mammary tumour) was reduced by 90% after 4 days of 5 mM DCA treatment, whereas a non-cancerous cell line was unaffected. There was no increase in caspase 3/7 activity, suggesting that growth inhibition is via decreased proliferation, rather than increased apoptosis. Growth inhibition was accompanied by decreased lactate production measured in the media after 24 hrs of DCA treatment, indicating that DCA is reaching its target and increasing pyruvate dehydrogenase activity via the inhibition of pyruvate dehydrogenase kinase.

In vivo, V14 cells were injected s.c. into syngeneic BALB/c mice and treated with 1.5 g/L DCA in the drinking water (~200 mg/kg/day). Growth of established V14 tumours was halted by DCA treatment, resulting in tumours being 28% smaller than the control group after 9 days of treatment (n=8 per group, p=0.08). Treatment from the day of cell injection (day 0) resulted in fewer tumours arising, and the appearance and regression of one tumour. Tumour lactate content was decreased 32% by DCA treatment. Histological assessment of the tumours found that DCA treatment resulted in an increase in tumour-associated lymphocytes and more fibrosis, suggesting a stronger anti-tumour immune response, consistent with regression. These results support the possibility that DCA could prevent or delay breast cancer formation in BALB/c-Trp53+/- mice, either through growth inhibition or enhanced immune function. Two groups (control and DCA treated) of Trp53+/- mice (n=50 per group) are currently being monitored for tumour development over 18 months. A positive result will support the testing of this drug as a chemopreventive agent in people highly predisposed to developing cancer.


This research was supported by NHMRC 366787 R.D. Wright Career Development Award and a National Breast Cancer Foundation Novel Concept Award.
AN EARLY AUDIT OF THE UTILITY OF BRAF TESTING IN 3 VICTORIAN FCCs.

C. Smyth 1, F. Macrae 2, A. Boussioutas 3, M. Bogwitz 1,2, G. Mitchell 3, M. Harris 1,3

Southern Health Familial Cancer Centre, Royal Melbourne Hospital Familial Cancer Centre, Peter MacCallum Familial Cancer Centre.

Testing for the common V600E mutation in BRAF in colorectal tumour specimens can assist the clinical assessment as to whether an individual has HNPCC or not. The presence of a BRAF mutation suggests that the absence of MLH1 and or PMS2 in an individual’s bowel cancer is likely to be due to methylation of the MLH1 promoter rather than the presence of a germline mutation causing HNPCC.

BRAF testing of tumours was introduced in Victoria in early 2008. A total of 32 BRAF tests were requested in the subsequent 16 months. Twenty-nine colorectal cancers, two small bowel tumours and 1 endometrial cancer were tested for BRAF mutations.

The ages of the tested probands at first cancer diagnosis ranged from 26-75 with a median age of 64 yrs. Six tumours tested were diagnosed in patients 50 years of age or younger. Five patients belonged to Amsterdam positive families, and 18 patients met the revised Bethesda criteria.

Results: 18 tumours were found to have the V600E BRAF mutation, and 14 did not. Generally, where a BRAF mutation was identified, this was interpreted as lowering the clinical probability of HNPCC, and lead to a change in management (usually surveillance advice for the individual or family) and was useful to the management of the case. In some instances a positive result meant that further genetic testing was spared (unless such testing had already occurred prior to availability of BRAF testing in January 2008). Generally, the absence of a mutation was interpreted as an increased likelihood of HNPCC and supported a decision to proceed to germline testing unless this had already occurred.

In 5 of the 10 individuals tested for V600E where the clinical impression of HNPCC was particularly low, (ie a single or two cases only of CRC over 60 and where MLH1/PMS2 loss on IHC had been found where such testing had been initiated by pathologists or others outside the FCC service), a BRAF mutation was identified.

Conclusion: This early data demonstrates some utility of BRAF testing, where the identification of the V600E mutation in approximately half of the cases tested was clinically useful.
SCREENING AND COUNSELLING ISSUES FOR MEMBERS OF FAMILIES AT HIGH-RISK OF PANCREATIC CANCER

Michelle Howson¹, Vu Kwan², Judy Kirk¹, Edwina Rickard¹ and Annabel Goodwin¹
¹Familial Cancer Service, Westmead Hospital
²Department of Gastroenterology and Hepatology, Westmead Hospital

Survival following a diagnosis of pancreatic cancer is low with a five-year survival rate of six per cent for males and eight per cent for females.¹ Endoscopic ultrasound (EUS) offers a minimally invasive method of imaging fine details in the pancreatic parenchyma that are not visible with conventional CT scan or transabdominal ultrasound. These features (including cystic lesions and changes of early chronic pancreatitis) have been shown to correlate with a spectrum of premalignant and malignant lesions at surgical histology. There are encouraging results from trials of pancreatic cancer screening for high-risk individuals using EUS with detection of precancerous lesions (pancreatic intraepithelial neoplasia, PanIN).²³ The International Symposium of Inherited Diseases of the Pancreas advises screening in accordance with current research protocols for high-risk individuals.⁴

We identified individuals at potentially high-risk of pancreatic cancer who have attended the Familial Cancer Service (FCS) by a systematic database search and file review. We defined individuals as being at potentially high-risk as:

- First-degree relatives of affected individuals with Familial Pancreatic Cancer (FPC) (defined as ≥ 2 first-degree relatives affected with pancreatic cancer).
- Peutz-Jeghers Syndrome (PJS) mutation carriers in families with a carrier/s affected by pancreatic cancer.
- BRCA2 mutation carriers in families with a carrier/s affected by pancreatic cancer.

Screening is advised from age 40 in all such cases.

Our search identified seven families. Within these families we identified 13 FCS patients fitting the above criteria. All 13 were contacted and advised EUS screening was an option. Uptake of a referral to a gastroenterologist to discuss EUS was high (62%). The small number of individuals referred meant it was feasible for the gastroenterologist to offer screening. Preliminary results will be presented.

Identifying and referring appropriate individuals combined with support from a gastroenterologist has allowed screening for pancreatic cancer to be offered to high-risk (PJS, BRCA2) or potentially high-risk (FPC) individuals. Strengthening the link between gastroenterology and the FCS may allow future involvement in international research collaborations, to develop evidence based screening protocols for a cancer that is usually advanced at presentation and difficult to treat.

Programme

Friday 14th August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 8a: Plantation Room

Chairperson: Melissa Southey
A GENOME-WIDE ASSOCIATION STUDY IDENTIFIES A NOVEL OVARIAN CANCER SUSCEPTIBILITY LOCUS ON 9p22.2

Simon A. Gayther 1, Paul D.P. Pharoah 2, Honglin Song 2, Susan J Ramus 1 and The Ovarian Cancer Association Consortium

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Women with a single first-degree relative diagnosed with epithelial ovarian cancer have a three-fold increased risk of developing the disease. Both environmental and genetic factors could contribute to this increased risk; but studies of twins suggest that genetic factors play a greater role. The known ovarian cancer susceptibility genes, including the highly penetrant BRCA1 and BRCA2 genes, explain less than half of the excess familial risk suggesting that other susceptibility genes exist. It is likely that the residual familial risks are due largely to a combination of several common and/or rare genetic variants in the population that confer more moderate to low penetrance susceptibility. In order to identify common alleles associated with ovarian cancer risk, we performed a genome wide association study (GWAS). We evaluated 507,094 SNPs that were successfully genotyped in 1,819 cases and 2,353 controls from the UK and the genotypes of ~2 million additional SNPs that were imputed from HapMap. We then genotyped the 22,790 top ranked SNPs in 4,833 cases and 5,237 controls from Europe, USA and Australia. We identified 12 SNPs that were associated with reduced disease risk with P<10^{-8}; all were from the same locus on 9p22. The most significant SNP (rs3814113; P = 6.9 x 10^{-18}) was genotyped in a further 2,962 ovarian cancer cases and 5,232 controls confirming its association (combined data odds ratio = 0.82 95% CI 0.79 – 0.86, P_trend = 9.9 x 10^{-20}). We also found that the association for rs3814113 differs by histological subtype, being strongest for serous ovarian cancers (OR 0.78 95% CI 0.71- 0.81, P_trend = 5.4 x 10^{-22}). Thus, rs3814113 is associated with a decrease in the risk of ovarian cancer in carriers of the minor allele. rs3814113 is not located within an open reading frame or an intronic region of any gene. The nearest gene is BNC2 (basonuclin 2), which encodes a DNA-binding zinc-finger protein suggesting it is an important regulatory protein for DNA transcription. The gene exhibits extensive transcriptional variability; it has six promoters and has the potential to generate up to 90,000 mRNA isoforms encoding more than 2,000 different proteins. BNC2 is highly expressed in reproductive tissues (ovary and testis) and may play a role in the differentiation of spermatoza and oocytes. Expression analysis in ovarian cancer cell lines and normal ovarian epithelial cells shows that BNC2 is down regulated in ovarian cancer cell lines (P = 0.0005). Germline methylation analysis in lymphocytes from ovarian cancer cases suggests that there is differential methylation associated with rs3814113 genotypes (P = 013). Additional studies aimed at fine mapping and functional analysis of the 9p22.2 locus to identify the causal susceptibility variant, and to identify additional ovarian cancer susceptibility loci will be discussed.
DISTRIBUTION OF BREAST CANCER ASSOCIATED SNPS IN CASES AND CONTROLS FROM THE AUSTRALIA BREAST CANCER FAMILY STUDY (ABCFS)

Wee Loon Ong, John Hopper, Georgia Chenevix-Trench, ABCFS, David Goldgar, Melissa Southey.

Background: Recent genome-wide-association-studies (GWASs) investigating common genetic variants have successfully identified single nucleotide polymorphisms (SNPs) in a number of independent loci to be associated with breast cancer risk. Each SNP confers only a small increase in breast cancer risk (per-allele-OR<1.5), but the SNPs are purported to act multiplicatively, giving a higher risk in individuals carrying multiple susceptibility SNPs.

Methods: Samples for the study were obtained from the ABCFS. Families with pathogenic BRCA1/2 mutations were excluded. DNA samples were extracted from Guthrie card blood spots, PCR-amplified using SNP-specific probes (Taqman), followed by end-point genotype analysis. 9 SNPs (verified by the Breast Cancer Association Consortium (BCAC)) have been selected for the study, namely rs2981582 (FGFR2), rs3803662 (TNRC9), rs3817198 (LSP1), rs889312 (MAP3K), rs13281615 (8q24), rs2107425 (H19), rs17468277 (CASP8), rs13387042 (2q35) and rs10941679 (5p12). For each individual, two risk scores were computed – total number of risk alleles (TOTRA) and total log odds ratio (LOGOR) as reported by BCAC.

Results: Genotypes for all 9 SNPs were available for 2572 individuals: 1031 case probands, 562 control probands and 979 first degree female relatives (mothers and sisters) of case probands, of whom 60 had breast cancer. The average age of breast cancer diagnosis for the case probands was 43, compared with 58 for the affected relatives. As expected, cases differed significantly from controls for both the LOGOR and TOTRA scores (p=0.000014 and p=0.0000034, respectively), with cases carrying on average 0.48 more risk alleles than controls. The highest quartile of the LOGOR score was associated with increased breast cancer risk compared to the lowest quartile (OR=1.55, p=0.003). Surprisingly, there was no difference in either score between the relatives as defined by their breast cancer status. In a Cox proportional hazards analysis of the risk of developing breast cancer in the relatives, neither SNP score significantly influenced the risk perhaps due to the small number of affected relatives. The correlation between probands’ and mothers’ LOGOR score was 0.36 (p<0.0001) and the intraclass correlation within sibships was 0.51 (p<0.0001) similar to that expected for an additive pure genetic trait. Our results call into question the utility of SNP-based risk prediction, even in the familial setting, although the addition of many more such SNPs may improve their utility.
THE USE OF PREDICTIVE OR PROGNOSTIC GENETIC BIOMARKERS IN ENDOMETRIAL AND OTHER HORMONE RELATED CANCERS: JUSTIFICATION FOR EXTENSIVE CANDIDATE GENE SNP STUDIES OF THE MATRIX METALLOPROTEINASE FAMILY AND THEIR INHIBITORS IN THE ERA OF GENOME-WIDE ASSOCIATION STUDIES.

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The emergence of genome-wide association studies (GWAS) have rapidly progressed the discovery of single nucleotide polymorphisms (SNPs) associated with susceptibility to complex diseases, including many malignancies. The matrix metalloproteinase (MMP) family of proteases and their tissue inhibitors (TIMPs) are involved in many cell processes, most notably the degradation of the extracellular matrix. Differences in gene and protein expression have been reported to be associated with many cancers and, in addition, increased expression of MMPs is frequently reported to be associated with poor prognostic features. We are embarking on a comprehensive study to assess the relevance of MMP/TIMP expression and MMP/TIMP SNPs in predisposition and prognosis of endometrial cancer, a largely hormonally driven cancer. An extensive literature review of the hormone related cancers of the prostate, breast and ovary showed that 62/69 studies assessing MMP expression in relation to prognosis reported upregulation of MMPs to be associated with poor prognostic features (grade, stage or survival). An extensive review of candidate studies of MMP/TIMP SNPs in hormonal cancers confirmed that these have generally been conducted on very small sample sizes, explaining why results for a given SNP may vary between studies. Nevertheless, there is some evidence to suggest that functionally relevant SNPs are associated with hormone related cancer prognosis and/or predisposition. No MMP or TIMP SNPs have been identified to be associated with cancer in GWAS published to date, but there are no reports assessing the proportion and the tagging efficiency of MMP/TIMP SNPs covered by high-throughput genotyping chips. A comprehensive search of multiple databases was conducted to identify the total number of known SNPs harboured by the MMP and TIMP gene families. The coverage of these genes by GWAS techniques was investigated using the Illumina Human 610-Quad Chip marker list. SNPs in high linkage disequilibrium were identified using genotype data available from the HapMap Database, and analysed using the Tagger and Haploview programs. The GWAS coverage of all MMP and TIMP genes was demonstrated to be very low, justifying the value of further candidate gene SNP studies of these genes. Given the large number of genes in these families (23 MMP and 4 TIMPS), methods to further prioritise SNPs for candidate gene studies have been investigated, including literature searches, bioinformatic and microarray analyses. Results from the investigation of the MMP/TIMP GWAS coverage and results from prioritization methods to identify most biologically relevant candidate genes for further SNP studies will be presented.
Programme

Thursday 13\textsuperscript{th} August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 8b: Plantation Room

Chairperson: Melanie Price
ADAPTATION TO LIVING WITH A FAMILIAL CANCER OR CANCER RISK

Barbara Biesecker, M.S., CGC National Human Genome Research Institute, NIH

Familial cancer and its inherent risks pose a significant health threat. As family members become aware of the threat to themselves or their close relatives, they appraise what it will mean for their lives. How they perceive the health threat is affected by prior expectations and experiences with cancer. Family members’ seek mastery or control over the cancer or cancer risk as they adapt to the threat. Adaptation refers to the process of coming to terms with the implications of the condition or risk and the observable outcomes of that process. How family members perceive the control they may have over the cancer or cancer risk influences the coping strategies they adopt. The effectiveness of their coping relates to how well the strategies address the practical and psychological needs generated by living with cancer or at risk for developing cancer. Over time, coping leads to adaptation to the condition or risk. We have developed an outcome measure of adaptation used in studies of children with pervasive developmental disorder, adults with Neurofibromatosis Type I, caregivers of children with Down syndrome, and adults at risk for Huntington disease. The measure includes sub-scales on self-esteem, psychological and spiritual well-being, coping outcomes and social integration and yields an overall measure of adaptation to the condition. Confirmatory factor analysis and reliability measures offer data to suggest that the sub-domains are well measured by the items and represent distinct non-overlapping constructs. This measure, developed in collaboration with the NIH Roadmap PROMIS consortium, may be used to assess adaptation in familial cancer families.
A QUALITATIVE STUDY OF HOW WOMEN AT HIGH BUT UNEXPLAINED FAMILIAL RISK OF BREAST CANCER PERCEIVE THEIR RISK

Keogh L, McClaren B, Apicella C, Hopper J for the Australian Breast Cancer Family Study

Background: Research on women’s perceptions of their risk of breast cancer has reported that most women over-estimate their risk. It has also been found that risk perception influences screening behaviour. However, there is a large amount of variation in the findings reported in the literature with perceived risk being positively associated with screening in some studies, but not others. We propose that conflicting findings may reflect inadequate attention to the meaning that risk holds for individual women.

Aim: To determine how women at high familial risk of breast cancer not due to carrying a mutation in BRCA1 or BRCA2 perceive their risk of breast cancer.

Methods: Since 1992, the Australian Breast Cancer Family Study (ABCFS) has been studying a large group of women who have had breast cancer, their relatives, and women who have not had cancer and their relatives. Participants have donated a blood sample for genetic research and answered survey questions. Women who have not had a mutation identified, but who are at a moderately increased familial risk of breast cancer (according to NBOCC guidelines) were invited to take part in this study. The data collection consisted of an audio recorded, semi-structured interview on the topic of breast cancer risk and screening decision-making in the area of cancer prevention. Data was analysed thematically to identify the different types of screeners, how risk was perceived, and the associations between these and other themes.

Results: A total of 24 interviews were conducted, and saturation of the main themes has been achieved. Based on women talking about their personal risk of cancer, they could be classified into four groups: (i) they thought they were unlikely to get breast cancer, (ii) they thought their cancer risk was above average, (ii) they thought cancer was inevitable for them, or (iv) they did not think about cancer, and therefore could not describe their personal risk. We consider how these perceptions of risk affected their screening behaviour.

Conclusions: These categories of risk are very different to those recognised and used by clinicians and researchers. In order to communicate better with these women at high but unexplained familial risk, it is important to recognise the language and framework they use, and to try to understand how these perceptions inform their health and lifestyle decision-making.
A LONG TERM FOLLOW UP PROGRAM FOR BRCA1/2 AND MMR GENE MUTATION CARRIERS: PRACTICALITIES OF DEVELOPING AND RUNNING A PROGRAM AND INITIAL RESULTS.

Lucinda Hossack¹, Mary Shanahan¹, Joanne McKinley¹, Mary-Anne Young¹, Gillian Mitchell¹.¹Familial Cancer Centre, Peter Mac Callum Cancer Centre, East Melbourne.

There is increasing evidence regarding the long term ongoing needs for people at high hereditary cancer risk including cancer risk management and psychosocial issues. In 2007 the Familial Cancer Centre (FCC) at Peter Mac sought to research, develop and implement a sustainable and comprehensive long term follow up program for gene mutation carriers. Findings from a planned observation of international models of long term follow up programs and undertaking a qualitative study to identify the needs of our local population were used to develop the annual follow up program we have implemented at the Peter Mac FCC.

The follow up program utilises a questionnaire as the primary contact method to ascertain any family and personal cancer history updates, risk management practices (including risk reducing surgery uptake, chemoprevention use and hormonal data) psychosocial and support needs and to flag in interest in research participation. Patients were offered the choice of completing a paper or electronic questionnaire. The questionnaire responses are used to triage the need for further contact by a telephone call with a Genetic Counsellor (GC), Genetic Nurse (GN) or to a face to face appointment in the FCC. To inform future sustainability of the program, we collected data on the time taken to mail the questionnaires, triage the data, respond to patient enquiries and perform any follow up tasks, including entry of data into an electronic database.

- 446 questionnaires were sent to eligible patients in February 2009. At the time this abstract was submitted, 18 patients did not receive the questionnaire, due to an out of date address, and 1 person had died. Of the remaining 427, 273 responded (64%). The median age of responders was 50 years (22-91).
- 6 people opted out of completing the questionnaire. Of the remaining 267, 164 (61%) were triaged to no further contact necessary this year. 103 (39%) patients required further contact from the FCC; of these 51 (49.5%) were triaged to contact with a GC, 42 (40.8%) to a GN and 10 (9.7%) to a face to face appointment in a multidisciplinary clinic.
- The median time taken to triage a questionnaire was 3 mins (1-10 mins). Data regarding the time taken for other aspects of the program, resources required and other practicalities for implementing such a program will be presented.
- We also plan to present descriptive data regarding the reasons for further contact, follow up provided by the FCC (including both medical and psychosocial support), as well as initial results of reported cancer history and current risk management practices.

The findings from our data demonstrate our model to be an effective and sustainable way of conducting long term follow up of mutation carriers. This method allows for better utilisation of FCC resources by ascertaining patient needs prior to personal contact as well as allowing for identification of the appropriate method and personnel to address patient’s needs. A questionnaire format has allowed for a large amount of information to be collected compared with our previous opportunistic telephone-based program, improving our research and clinical audit capacity. Furthermore this data potentially allows for measures of the utility of annual review by this method through monitoring changes in risk management practices and psychosocial needs reported by patient’s overtime.
NON-ACCEPTANCE OF AN APPOINTMENT AT THE FAMILIAL CANCER CLINIC: A PRELIMINARY TELEPHONE SURVEY.

K Ryan, J Armstrong, M Raciti, S Russell, G Suthers, D Trott, NK Poplawski

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**Aim and Methods:** To explore the reasons why some clients who are referred to our service do not respond to our pre-clinic contact letter requesting they complete and return a series of pre-clinic forms (PCFs), despite being informed that an appointment will only be arranged if the PCFs are returned.

**Study population:** A group of referred clients who failed to return their PCFs more than 5 months after receiving them were identified (N=64). Up to 3 separate attempts were made to contact each client by phone. 47/64 were able to be contacted and were surveyed; answers were recorded and coded on a one-page questionnaire.

**Results:** The 47 non-responders surveyed gave 57 reasons for not returning their PCFs. More than half of the reasons related to difficulties filling out the family history form (N=35; 61%). Other reasons included; an appointment not being a current priority because of issues related to ongoing cancer treatment (N=4); concerns about genetic information (N=4); not understanding why a referral was made (N=2); recent death of a close relative (N=2); and concern about family confidentiality (N=1).

More than 2 thirds (30; 63%) of non-responders indicated they wanted an appointment “now” and would return their PCFs, although less than half (13; 43%) have returned them in the 4-6 month period since we contacted them. Difficulties filling out the family history forms were reported by almost all (28; 93%) of this subgroup.

Just over 1 third of non-responders (N=17; 36%) indicated they would not return the PCFs; 11 did not want an appointment and 6 did not want an appointment “now” but would consider re-referral at a later date. Difficulties filling out the family history forms were reported by 7 (41%) of this sub-group. Of interest, the PCFs have been returned by 1/11 who indicated they did not want an appointment and 4/6 who were considering being seen at a later date.

**Conclusion:** Almost 2 in 3 non-responders want to be seen at an appointment but have not returned their PCFs, primarily because of difficulties with the family history form. We need to explore ways to assist completion of these forms whilst minimising the impact on Genetic Counsellor workload. As a proportion of non-responders are passively indicating their decision not to have an appointment with the FCU, any attempts to improve form return rates should show respect regarding the decision of this subgroup.
Programme

Friday 14th August

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Session 9: Plantation Room

Chairperson: Georgia Chenevix-Trench
A TALE OF TWO BRCA1 SEQUENCE VARIANTS: EVIDENCE FOR INTERMEDIATE FUNCTION AND INTERMEDIATE CANCER RISK.

Spurdle AB1,2, Brown MA2, Pettigrew C2, Thompson B1, Healey S1, kConFab3, Benitez J4, Borg A5, Campbell J5, Watts S6, Eccles D7, Sinilnikova O8, Toland A9, Van Overeem Hansen T10, Vreeswijk M11, Devilee P11, Wappenschmidt B12, Engel C13, Tavtigian SV14, Couch FC15, Goldgar DE16

1Queensland Institute of Medical Research, Brisbane, Australia; 2University of Queensland, Brisbane, Australia; 3Peter MacCallum Cancer Centre, Melbourne, Australia; 4Spanish National Cancer Centre, Madrid, Spain; 5Lund University, Lund, Sweden; 6Guy's Hospital, London, UK; 7Wessex Clinical Genetics Service, Southampton, UK; 8Universite Lyon, Lyon, France; 9Ohio State University Comprehensive Cancer Centre, Columbus, USA; 10Rigshospitalet, Copenhagen, Denmark; 11Leiden University Medical Center, Leiden, The Netherlands; 12University of Cologne, Germany; 13University of Leipzig, Germany; 14International Agency for Research on Cancer, Lyon, France; 15Mayo Clinic College of Medicine, Rochester, USA; 16University of Utah School of Medicine, Salt Lake City, USA

The clinical classification of rare sequence variants identified in the breast cancer susceptibility genes BRCA1 and BRCA2 is essential for appropriate genetic counselling of individuals carrying these variants. Multifactorial likelihood analysis methods and functional assays are both used to elucidate likely clinical significance of such variants. We recently conducted a study of several unclassified variants located in the BRCA1 transactivation domain (TAD), comparing the results from multifactorial likelihood analysis for with those from several functional assays. Our initial results raised the possibility that a subset of these rare BRCA1 variants might be associated with intermediate function and this could perhaps also be associated with an intermediate cancer risk. In the present study, we focused on variants reported across different studies in the literature to have intermediate transcriptional transactivation activity, and re-assessed their activity in controlled experiments in a single laboratory, comparing to known pathogenic control mutations. Our results indicate that TAD assay results can vary markedly depending on the host cell lines used for transfection, and provide additional evidence that R1699Q, A1708V, V1804D and L1564P can demonstrate significantly lower activity relative to wildtype BRCA1. To see if this translated into lower genetic risk, we initiated a large collaborative study to obtain a large sample of pedigrees for one such variant, R1699Q. As a control, we collected pedigree and genotype data from the same centres for the variant R1699W, which is known to be pathogenic and is similar in TAD function to null mutations. A series of genetic analyses were carried out comparing R1699Q to R1699W. There was evidence for a difference in reported family history between 38 R1699Q families and 30 R1699W families. Manchester scores were significantly different, while Myriad logistic regression analysis showed a similar, but non-significant, trend, with R1699W having higher predicted BRCA1 probabilities. The combined Bayes odds in favour of causality were 1145:1 for the 21 informative R1699Q families, and 671000:1 for 14 informative R1699W families. Compared to reference pathogenic BRCA1 mutation penetrance estimates, the best estimate of relative penetrance was 0.6 for R1699Q and 1.1 for R1699Q, but this difference was not statistically significant. While the Bayes odds of > 1000:1 for R1699Q would be interpreted as indicative of pathogenicity, comparison of a number of different features to a reference missense pathogenic mutation at the same residue suggest that this variant does not exhibit the profile of a classical high-risk BRCA1 mutation. Current multifactorial likelihood approaches require development to account for probable moderate-risk variants.
RARE, EVOLUTIONARILY UNLIKELY MISSENSE SUBSTITUTIONS IN ATM CONFER INCREASED RISK OF BREAST CANCER

Sean V. Tavtigian,1* Peter J. Oefner,2* Anne Hartmann,2 Sue Healey,3 Florence Le Calvez-Kelm,1 Fabienne Lesueur,1 Davit Babikyan,1 Graham B. Byrnes,1 Shu-Chun Chuang,1 Nathalie Forey,1 Corinna Feuchttinger,2 Lydie Gioia,1 Janet Hall,4 Mia Hashibe,1 Barbara Herte,2 Sandrine McKay-Chopin,1 Alun Thomas,5 Catherine Voegele,1 Penelope M Webb,3 David C Whiteman,3 Australian Cancer Study, BCFR, kConFab,6 Suleeporn Sangrajrang,7 John L. Hopper,8 Melissa C. Southey,8 Irene L. Andrulis,9 Esther M. John,10 Georgia Chenevix-Trench.3

1. International Agency for Research on Cancer, 69372 Lyon, France. 2. Institute of Functional Genomics, University of Regensburg, 93053 Regensburg, Germany. 3. Queensland Institute of Medical Research, Brisbane, Australia. 4 Institut Curie - Recherche; INSERM U612; 91405 Orsay, France. 5 Department of Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, UT, USA. 6 Peter MacCallum Cancer Centre, Melbourne, Australia. 7 Research Division, National Cancer Institute, Bangkok, Thailand. 8 Centre for MEGA Epidemiology, University of Melbourne, Carlton, Victoria, Australia. 9 Cancer Care Ontario, Fred A. Litwin Center for Cancer Genetics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5 Canada. 10 Northern California Cancer Center, Fremont, CA, USA and Department of Health Research and Policy, Stanford University School of Medicine, Stanford, CA, USA.

* These authors contributed equally to this work.

The susceptibility gene for Ataxia telangiectasia, ATM, is also an intermediate-risk breast cancer susceptibility gene. However, the spectrum and frequency distribution of ATM mutations that confer increased risk of breast cancer have been controversial. To assess the contribution of rare variants in this gene to risk of breast cancer, we pooled data from seven published ATM case-control mutation screening studies including a total of 1544 breast cancer cases and 1224 controls with data from our own mutation screening of an additional 987 breast cancer cases and 1021 controls. Using an in silico missense substitution analysis that provides a ranking of missense substitutions from evolutionarily most likely to least likely, we carried out analyses of protein truncating variants, splice junction variants, and rare missense variants. We found marginal evidence that the combination of ATM protein truncating and splice junction variants contribute to breast cancer risk. There was stronger evidence that a subset of rare, evolutionarily unlikely, missense substitutions confer increased risk. On the basis of subset analyses, we hypothesize that rare missense substitutions falling in and around the FAT, kinase, and FATC domains of the protein may be disproportionately responsible for that risk, and that a subset of these may confer higher risk than do protein truncating variants. We conclude that a comparison between the graded distributions of missense substitutions in cases versus controls can complement analyses of truncating variants to help identify susceptibility genes, and this approach will help interpret the wealth of data emerging from new sequencing technologies.
ASSIGNING BREAST CANCER RISKS TO WOMEN WITH UNCLASSIFIED VARIANTS IN BRCA1

JG Dowty1, E Lee2, G Ursin2,3, JL Hopper1.

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3Department of Nutrition, University of Oslo, Norway

A number of measures have been proposed to predict the “pathogenicity” of otherwise unclassified variants (UVs) in the breast and ovarian cancer susceptibility gene BRCA1. We assess the effectiveness of four of these measures using data from a US population-based study of 1469 early-onset breast cancer cases (probands) and their first- and second-degree relatives. The probands’ BRCA1 and BRCA2 genes were sequenced and 299 families segregating a BRCA1 UV, and not segregating a BRCA2 mutation, were identified. These UVs were classified into ‘high’ and ‘low’ risk categories using polyphen (PP), Grantham matrix scores (GMS), sequence conservation (SC) and a combination of sequence conservation and Grantham matrix scores (SCGMS). For each of the four UV classification measures, the hazard ratio (HR = the age-specific incidence of breast cancer for carriers of a UV designated as ‘high’ risk divided by the corresponding population incidence rates) was estimated using modified segregation analyses.

The estimated HRs were: 3.7 (95% CI 0.9-15.3, p=0.14) for PP; 2.0 (95% CI 0.6-6.9, p=0.11) for GMS; 5.7 (95% CI 1.5-22.5, p=0.05) for SC; and 4.9 (95% CI 0.6-43.2, p=0.30) for SCGMS. That is, women carrying a UV in the SC high-risk category are estimated to have incidence of breast cancer roughly 6 times higher than that of the general population. More detailed analyses of the SC measure showed that, above a certain threshold, there was an increase in risk as the degree of sequence conservation increased (p=0.02 for trend).

These results suggest that UVs which are classified as ‘high’ risk by sequence conservation methods might confer substantial increases in breast cancer risk, though larger studies are needed to provide more precise estimates of the corresponding risks and to consider combinations of measures. This work also demonstrates that, as an alternative to trying to classify individual UVs as “pathogenic” or not, empirical approaches can be used to estimate the actual cancer risks associated with categories of UVs, just as a woman with a unique protein-truncating mutation is counselled based on the category.
DETECTABLE METHYLATION OF THE BRCA1 PROMOTER REGION IN PERIPHERAL BLOOD IS ASSOCIATED WITH RISK OF EARLY-ONSET BREAST CANCER WITH BRCA1 MUTATION ASSOCIATED TUMOUR MORPHOLOGY

Ee Ming Wong1,2, Australian Breast Cancer Family Registry, Stephen Fox1,2, Melissa A. Brown3, Mark A. Jenkins4, Margaret R.E. McCredie4, Andrea A. Tesoriero2, Letitia D. Smith2, John L. Hopper4, Graham G. Giles5, Melissa C. Southey2 and Alexander Dobrovic1,2

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5 Cancer Council of Victoria, Melbourne

The tumours that arise in BRCA1 mutation carriers usually have a number of distinctive morphological features. However, a substantial proportion of breast cancers that display these multiple features do not arise in known mutation carriers. As BRCA1 is subject to inactivation by promoter methylation, we hypothesised that some of these latter breast cancers might result from constitutional somatic methylation of the BRCA1 promoter region.

We studied three groups of women selected from a population-based case-control-family study of breast cancer; 56 cases with breast cancer diagnosed before the age of 40 years whose tumours had five or more of nine BRCA1 mutation-associated morphological features (group 1); 127 cases with breast cancer diagnosed before the age of 40 years whose tumours had less than five of these morphological features (group 2); and 123 age-matched unaffected women (group 3). All cases had undergone extensive BRCA1 mutation screening and no BRCA1 germline mutations had been identified. The methylation status of the BRCA1 promoter region was measured in DNA extracted from peripheral blood and microdissected tumour sections.

The prevalence of methylation in the peripheral blood DNA (at levels ranging from 0.1% to 13%) was 18/56 (32%) for Group 1. This was ~10-times higher than for Group 2 (5/123: 4%) and for Group 3 (4/127: 3%) (both p<10^-6). Tumour-enriched DNA from women in Group 1 who had detectable methylation in peripheral blood DNA were all heavily methylated but no tumour-enriched DNA sample from women in Group 2 had increased methylation relative to the peripheral blood. In conclusion, constitutional somatic methylation of the BRCA1 promoter region is associated with an increased risk of early-onset breast cancer. Given that about one-quarter of these early-onset breast cancers have at least five of the nine morphology features, then about 1 in 8 early-onset cases will be methylated, and the odds ratio of methylation for disease is 4.2. If methylation is causal, its population attributable fraction (PAF) is about 9%. 

“Familial Cancer 2009: Research and Practice”
Programme

Wednesday 12th

A combined meeting of kConFab, Australian Breast Cancer Family Study, Australasian Colorectal Cancer Family Study, Australian Ovarian Cancer Study, Family Cancer Clinics of Australia and New Zealand & kConFab

“Familial Cancer 2009: Research and Practice”

Poster Session
7.30 – 10.30pm
DO FAMILIES WANT CANCER GENETIC TESTING? YES THEY DO!

Annabel Goodwin¹, Jenny Leary¹, Barb Guild¹ and Judy Kirk¹
¹Familial Cancer Service, Westmead Hospital

The majority of families with a family history of breast and/or ovarian cancer attending the Familial Cancer Service (FCS) have not previously had any genetic testing. The uptake of testing (mutation search) for families where genetic testing was appropriate was reviewed.

From 1994 to February 2009, 1041 families who fulfilled the National Breast and Ovarian Cancer Centre’s potentially high risk category (Category 3) were offered a mutation search of BRCA1/2.

Some of the 1041 could not proceed with testing since there was no living affected relative (10%) or no contact with an affected relative (3.1%). For the remaining 905 families, an affected proband first attended the FCS clinic in 560 families and an unaffected relative attended for the remaining 345. Those unaffected relatives were advised that an affected family member could be offered a mutation search. Uptake was highest (89.5%) when the affected family member first attended the clinic. When unaffected relatives attended, testing subsequently occurred for 31.6% at either the Westmead FCS or at another service. Overall, where testing was possible, 67.4% of families proceeded with testing.

For the 905 families, the uptake of testing for kindreds with breast cancer only was 65% - of those with results, a pathogenic mutation was identified in 9.1%. Uptake of testing for families with breast/ovarian cancer was 76.1% - a mutation was identified in 36.9%. Uptake of testing for families with ovarian cancer only (n=5) was 80% - a mutation was identified in 25%.

An additional 67 individuals (both affected and unaffected) from 60 families were offered a limited mutation search of BRCA1/2 for the Ashkenazi Jewish founder mutations. Uptake was 88.1% and a mutation was identified in 6.8%.

Uptake of mutation search in families where genetic testing is appropriate and possible is high. In non-Jewish families the overall uptake was 67.4%, but higher if an affected family member attended the FCS (89.5%). Families are keen to take up the opportunity of genetic testing, but may find that approaching other relatives to participate in testing is difficult.
GENETIC REFERRALS FROM GYNAECOLOGICAL ONCOLOGY MULTIDISCIPLINARY TEAMS – REVIEW AND OUTCOMES.

Jessica Koehler, Janet Tyler, Dr Kathy Tucker, Dr Lesley Andrews
Prince of Wales Hereditary Cancer Clinic

Multidisciplinary care is now routine practice in oncology units in Australia in order to standardise cancer care. To encourage new referrals to the Hereditary Cancer Clinic, staff have been attending the weekly Gynaecological Oncology multidisciplinary meeting at the Royal Hospital for Women since 2007.

A review of 717 cases of women with gynaecological malignancy presented in 2007-2008 was conducted. 101 of the cases presented indicated referral for hereditary cancer assessment. 39 had no known family history of cancer but suggested referral due to age or multiple primary cancers or pathology type (eg primary fallopian tube cancer). Referral and uptake of genetics appointments was low, with only 43 women having attended the clinic.

Immunohistochemistry stains for MLH1, MSH2, MSH6 and PMS2 were organised for 16 tumours, three were indicative of Lynch Syndrome and had mismatch repair mutations identified. In addition, 22 BRCA1/2 mutation tests were completed, identifying four BRCA1 and three BRCA2 mutations.

Genetic attendance at the gynaecological tumour board meeting resulted in increased referrals although many patients declined the offer of an appointment. To address the low uptake of referrals, all patients for whom referral is indicated are now either seen on the ward following their surgery or contacted via letter to introduce the service. All early onset invasive endometrial (diagnosed <50) and ovarian (diagnosed <40) tumours will now undergo mismatch repair immunohistochemistry as standard practice to better triage presumptive Lynch Syndrome referrals.
NEEDS ASSESSMENT OF WOMEN UNDERGOING RISK REDUCING SALPINGO-OOPHORECTOMY.

1Mary Shanahan, 1Carmel Pezaro, 1Mary-Anne Young, 1Joanne McKinley, 1Gillian Mitchell
1Peter MacCallum Cancer Centre

Background
Women in families with the hereditary breast and ovarian cancer syndrome due to germ line mutations in BRCA1 and BRCA2 genes are at increased risk of developing breast and/or ovarian cancer. Current screening methods are ineffective for ovarian cancer. Consequently a risk reducing bilateral salpingo-oophorectomy (RRBSO) is the most effective means of managing the ovarian cancer risk in this setting. There is limited data about the acute consequences of a RRBSO in either the pre or postmenopausal setting and even less about the long-term effects.

Aims:
1 Assess the specific needs of a cohort of women with germline BRCA1 or BRCA2 mutations undertaking RRBSO.
2. Create a cohort to assess prospectively the full spectrum of the long-term consequences of premenopausal RRBSO.

Methods
Women were invited to participate and sent a questionnaire. The questionnaire was designed to establish what follow up programs were in place post-operatively and specifically what arrangements were in place to monitor and manage bone density. Menopausal symptoms were established using a validated menopause rating scale which was designed to assess the severity of the symptoms. Women were also offered the opportunity to express an interest in participating in future focus groups about this topic.

Results
• Three hundred and fifty eight female BRCA1 and BRCA2 mutation carriers were identified. One hundred and thirty-three were excluded because they were known to have their ovaries in tact or were not suitable for contact.
• Questionnaires were sent to 225 and a response was received from 172 (76%). 17 (8%) were uncontactable, 13 (6%) opted out and 23 (10%) did not respond.
• Of the responders, 156 (91%) had undergone bilateral oophorectomy and 16 (9%) still had their ovaries in situ.
• Severity of menopausal symptoms and details of follow up after RRBSO will be reported.

Conclusion
The results of this study will be used to develop a coordinated approach to the management of women who undergo a risk reducing salpingo-oophorectomy.
A QUALITATIVE STUDY OF HOW WOMEN AT HIGH BUT UNEXPLAINED FAMILIAL RISK OF BREAST CANCER PERCEIVE THEIR RISK

Keogh L, McClaren B, Apicella C, Hopper J for the Australian Breast Cancer Family Study

Background: Research on women’s perceptions of their risk of breast cancer has reported that most women over-estimate their risk. It has also been found that risk perception influences screening behaviour. However, there is a large amount of variation in the findings reported in the literature with perceived risk being positively associated with screening in some studies, but not others. We propose that conflicting findings may reflect inadequate attention to the meaning that risk holds for individual women.

Aim: To determine how women at high familial risk of breast cancer not due to carrying a mutation in BRCA1 or BRCA2 perceive their risk of breast cancer.

Methods: Since 1992, the Australian Breast Cancer Family Study (ABCFS) has been studying a large group of women who have had breast cancer, their relatives, and women who have not had cancer and their relatives. Participants have donated a blood sample for genetic research and answered survey questions. Women who have not had a mutation identified, but who are at a moderately increased familial risk of breast cancer (according to NBOCC guidelines) were invited to take participate in this study. The data collection consisted of an audio recorded, semi-structured interview on the topic of breast cancer risk and screening decision-making in the area of cancer prevention. Data was analysed thematically to identify the different types of screeners, how risk was perceived, and the associations between these and other themes.

Results: A total of 24 interviews were conducted, and saturation of the main themes has been achieved. Based on women talking about their personal risk of cancer, they could be classified into four groups: (i) they thought they were unlikely to get breast cancer, (ii) they thought their cancer risk was above average, (ii) they thought cancer was inevitable for them, or (iv) they did not think about cancer, and therefore could not describe their personal risk. We consider how these perceptions of risk affected their screening behaviour.

Conclusions: These categories of risk are very different to those recognised and used by clinicians and researchers. In order to communicate better with these women at high but unexplained familial risk, it is important to recognise the language and framework they use, and to try to understand how these perceptions inform their health and lifestyle decision-making.
GENETIC COUNSELLING FOR FEMALE BRCA CARRIERS IN MALAYSIA INCREASES UPTAKE OF CANCER SURVEILLANCE

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Cancer Research Initiatives Foundation, Malaysia¹, University of Malaya Medical Centre² Email: sookyee.yoon@carif.com.my

To date, there are no familial cancer services in Malaysia, but since July 2007, we have provided genetic testing and genetic counselling services by qualified professionals in research settings. As part of a University of Malaya and CARIF’s ongoing research to identify genetic loci that predispose women to breast cancer, a multi-disciplinary team comprising of a clinical geneticist, an associate genetic counsellor, a breast surgeon and a laboratory scientist were involved in counselling patients and their families in this pilot programme.

Forty BRCA carriers who have had breast cancer and 5 unaffected female BRCA carriers were provided with genetic counselling and results disclosure. The median age of affected BRCA carriers was 45 years (range 24 to 64) and the median age of unaffected BRCA carriers was 41 years (range 25 to 54). The carriers comprised 23 Chinese, 12 Malays and 10 Indians. During the counselling session, clients were informed about their increased risk to contralateral breast cancer and ovarian cancer. Approximately 25% of the counselling session was used for discussion on options available for risk management, including screening and prophylactic surgery.

We report that of the 40 affected BRCA carriers, 37 (93%) were already on a regular follow-up surveillance plan involving a clinical examination and 6 monthly mammogram for breast cancer and were diligent in adhering to the plan. After genetic counselling, 100% adherence was achieved with a median follow up period of 15 months (1 to 25 months) because of awareness that they may have recurrence and post-genetic counselling, they were aware of the increased risk for contra-lateral breast cancer. Unfortunately, 10 carriers developed contralateral breast cancer, of whom 4 carriers developed contralateral breast cancer post-results disclosure. Notably, and in sharp contrast to experiences in more established familial cancer clinics, only 2 carriers (5%) have opted for prophylactic mastectomy and none have chosen chemoprevention.

Of the 40 affected BRCA carriers, only 7 (17%) have ever had a gynaecological screen prior to genetic counselling and testing. All 7 patients had gynaecological consultation for unrelated problems, such as unrelated problems in the ovaries or PAP smears. After genetic counselling, 3 carriers (8%) decided to have prophylactic oophorectomy and 27 (68%) have started on an ovarian screening programme using 6 monthly CA125 and transvaginal ultrasound. Four patients have passed away and 6 patients have not yet taken screening or surgery. Significantly, 36 patients (90%) were unaware of their increased risk to ovarian cancer prior to genetic counselling.

For the 5 unaffected BRCA carriers, only 1 (20%) had conducted breast self-examination or has had clinical breast examination, despite having high risk families with multiple cases of breast or ovarian cancer. However, after genetic counselling of their genetic status and risks, all 5 (100%) have started breast and ovarian surveillance.

CONCLUSION: Genetic counselling in Malaysia helps to provide relevant risk and cancer surveillance information to BRCA carriers and the genetic information provides strong motivators for uptake of appropriate risk management strategies. However, our experience with this unique cohort of Asians indicates that the uptake of prophylactic surgery is lower than that reported in other populations and that most patients prefer surveillance programmes.
ANALYSIS OF TP53 GERMLINE MUTATIONS IN ASIAN EARLY ONSET BREAST CANCER PATIENTS

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INTRODUCTION: Mutations in TP53 are rare and can lead to very high breast cancer risk, but to date, the contribution of TP53 has not been systematically studied in Asians. Given that most breast cancers associated with mutations in TP53 (Li Fraumeni or Li-Fraumeni-like syndrome) are diagnosed before 50 and the mean age of diagnosis is in the early 30s⁵, we conducted a review of the prevalence of early onset breast cancer cases (<35 years old) and those with LFS or LFL characteristics in a hospital-based cohort of breast cancer patients in Malaysia, and examined the status of BRCA1, BRCA2 and TP53 in these individuals.

RESULTS:
University Malaya Medical Centre is a major tertiary referral centre in Kuala Lumpur Malaysia. In the past 16 years (1993 to Dec 2008), 3,438 breast cancer patients were treated at UMMC, of which 244 (7%) were diagnosed with invasive breast cancer under the age of 35 years old. Since the establishment of the MyBrCa (Malaysian Breast Cancer Genetic Study) in Jan 2003, 86 breast cancer patients who developed breast cancer <35 years old have taken part in the research study.

• BRCA1 and BRCA2 sequencing and MLPA analysis was completed in all 86 women and deleterious frameshift, nonsense or splice site mutations were detected in 15 individuals (17% in total; 9 BRCA1 and 6 BRCA2). Notably, the majority of individuals (11/15) had no reported family history of breast or ovarian cancer. Only 4 individuals were “high-risk” and had a combined Manchester score of 8 to11 respectively.
• Of the remaining 71 individuals, 14 had variants of uncertain clinical significance (VUS): 3 in BRCA1, 11 in BRCA2 and 2 in both genes. Of these 71 individuals, 36 had no reported family history of breast or ovarian cancer, 11 were moderate risk with Manchester score between 10 and 14 and 4 were “high-risk” with combined Manchester score of ≥15.
• Notably, only 6 individuals fulfilled the LFS/LFL family characteristics. Mutation analysis of TP53 is ongoing in these and all other individuals who developed breast cancer <35.

CONCLUSION: Early onset breast cancer constitutes a higher proportion of the breast cancer patients treated in this Asian setting, where 7% of patients developed breast cancer <35. Notably, the majority of these patients report no significant family history, thereby posing significant challenges in the selection of individuals who may benefit from genetic testing.

⁵ Olivier et al. (2003), Cancer Research 63, p6643
THE SYMPTOMATIC, METABOLIC AND PSYCHOSEXUAL CONSEQUENCES OF SURGICAL MENOPAUSE

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Surgical menopause refers to the removal of all ovarian tissue in a premenopausal woman\(^1\). The prevalence of surgical menopause in Australia is not known, although it is likely to be increasing. There is currently little evidence-based guidance on the advantages and disadvantages of bilateral oophorectomy in this age group. Further, the symptomatic and long-term health consequences of surgical menopause are poorly understood.

For those women carrying the BRCA 1 and 2 germline mutations the relative benefits of risk reducing BSO (RRBSO) is more clearly defined. These women carry a lifetime risk ovarian cancer of 10-46\(^2\) and current screening methods are ineffective in detecting tumours at a sufficiently early stage to influence survival\(^3\) The only intervention that has been shown to be effective in reducing the incidence of ovarian cancer in these women is BSO which reduces the risk of ovarian cancer by around 90%\(^5\) and may also reduce breast cancer risk when performed in younger women\(^4\)

The aim of the study is to prospectively determine the impact of surgical menopause on the nature, severity and duration of menopausal symptoms, quality of life, sexual function and bone health.

Premenopausal women (<45 years) who carry the BRCA1 or BRCA2 germline mutation planning to undergo surgical menopause will be recruited from public (King Edward’s Memorial Hospital, KEMH) and private clinics in Western Australia.

Bone turnover will be measured from standard fasting plasma measurements. A spot urine test will measure urinary calcium creatinine ratio, phosphate and N-telopeptide region (NTX). Bone density will be measured using DEXA scanning.

The authors hypothesize that surgical menopause will be associated with more severe vasomotor symptoms, a deterioration in sexual function and markers of increased bone turnover. All of these factors will become an important component of preoperative counselling for these high-risk patients.

References

A REVIEW OF PATIENTS DIAGNOSED WITH SYNCHRONOUS OR METACHRONOUS BREAST/OVARIAN CANCER REFERRED TO THE HEREDITARY CANCER CLINICS AT LIVERPOOL, ROYAL NORTH SHORE, ROYAL PRINCE ALFRED, ST GEORGE AND WOLLONGONG HOSPITALS BETWEEN 1999 AND 2009.

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Between 1999 and June 2009 40 patients diagnosed with either synchronous or metachronous breast/ovarian cancer attended the Hereditary Cancer Clinics at Liverpool, Royal North Shore, Royal Prince Alfred, St George and Wollongong hospitals. Mutation analysis of the BRCA1 and BRCA2 genes was offered to all patients (except 1 diagnosed with mucinous ovarian cancer); of which four patients declined or deferred testing. None previously had a genetic mutation identified in their family.

In this cohort of 35 patients, 11 BRCA1 and 3 BRCA2 mutations were identified, with an overall mutation detection rate of 40%. 7 of the remaining 21 probands (33%) had one or more unclassified variants identified in either BRCA1 or BRCA2. We have evaluated the carrier prediction algorithms BOADICEA, combined Manchester score and the NBOCC guidelines. Using a 10% BRCA1 or BRCA2 mutation detection threshold BOADICEA showed 100% sensitivity (14.2% specificity) and Manchester score (of $\geq 15$) 100% sensitivity (38% specificity). If a 20% threshold is applied BOADICEA showed 92.8% sensitivity (47.6% specificity) and Manchester score (of $\geq 20$) 92.8% sensitivity (61.9% specificity). Using the high risk category as per the NBOCC guide gave 85.7% sensitivity (52.3% specificity).

There were some key findings when comparing those patients with an identifiable BRCA1 or BRCA2 mutation to those without. Mutation carriers were diagnosed with breast cancer at a younger age, with a mean age of 43 years (range 27-59 years) compared to 58 years (range 45-72 years) in the non-carriers (p<0.0001). Mutation carriers were also diagnosed with ovarian cancer at a younger age, with a mean of age 54 years (41-73 years) compared to 62 years (range 46-77 years) in the non-carriers (p= 0.01). Almost half of the patients (5/11) with a BRCA1 mutation had been diagnosed with bilateral breast cancer. No mutations were identified in the 4 patients diagnosed with non-serous ovarian cancer and only one mutation was identified in the 6 patients diagnosed with non-invasive ductal breast cancer.

Over the past ten years patients with a diagnosis of breast and ovarian cancer have been routinely offered BRCA1 and BRCA2 mutation analysis regardless of family history. Whilst 2 mutations were identified in non-high risk families according to NBOCC guidelines, no mutations were identified in patients that did not meet a BOADICEA of 10% or combined Manchester score of 15 or over. This review provides insight into a small cohort of probands that have a high incidence of an underlying BRCA1 or BRCA2 mutation in the presence of young age of onset and/or a family history of breast/ovarian cancer.
#9
BRCA GENOTYPING IN THE AUSTRALIAN OVARIAN CANCER STUDY: AN UPDATE

Kathryn Alsop¹, Cliff Meldrum¹, Michael Birrer², Mary-Anne Young¹, the Australian Ovarian Cancer Study Group, Penny Webb³, Stephen Fox¹, Gillian Mitchell¹ and David Bowtell¹

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Identification of BRCA mutation carriers is a clinically important measure in prevention of ovarian cancer, but is hampered due to high costs of genetic testing in the public sector and the unreliability of using a strong family history of breast and ovarian cancer as the major selection criteria for testing. A Canadian population-based study has recently demonstrated a surprisingly high frequency of BRCA1/2 mutations in ovarian cancer cases, including among women who, on family history criteria, would not normally be considered to be at substantial risk of having such mutations. The Australian Ovarian Cancer Study (AOCS) has received funding from the US Department of Defence Ovarian Cancer Research Program Translation Research Partnership to genotype 1000 cases of invasive epithelial ovarian cancer for mutations in the BRCA1 and BRCA2 genes. Testing of AOCS participants is currently underway in collaboration with the Peter MacCallum Cancer Centre (PMCC) Pathology Department, using full genomic DNA sequencing and multiplex ligation-dependent probe amplification (MLPA). Clinically significant results will be returned to participants via a model similar to that in use by kConFab, and facilitated with the cooperation of the national Familial Cancer Centres (FCC).

Results: To date, 3 new mutation carriers have been identified amongst the first 26 cases to be screened at the PMCC, and a further 30 have been identified from the results of clinical testing through local FCCs. To identify and validate new subgroups of women considered “at-risk” of harbouring a BRCA mutation, and therefore to allow improved identification of carriers in the population, the genotyping information will be used in conjunction with pathological, clinical and new family history data. Family history questionnaires have been sent to 935 living AOCS case participants. Over 35% of the approached cohort has responded to date, and over 200 pedigrees have been compiled.

Future directions: Recent studies have shown that germline BRCA mutation status is prognostically significant. There is evidence to suggest that some ovarian cancers not associated with germline BRCA mutations can also display evidence of “BRCA-ness”; it is unknown whether this too is prognostically significant. We will use the genotype data to stratify a subgroup of approximately 170 serous ovarian tumours that have been expression arrayed, with the aim of identifying the molecular features of “BRCA-ness”, and correlate this with therapy outcomes. The contribution that disruption of the homologous recombination pathway makes in the sensitivity of ovarian tumours to current therapeutics will also be explored.
#10
BRCA1 AND BRCA2 MLPA TESTING IN VICTORIAN AND TASMANIAN FAMILIES WITH FAMILIAL BREAST/OVARIAN CANCER


**Background:** The current debate is not whether we should attempt to identify germline mutations in families at risk, but rather how we identify them, balancing the costs for the tests against the probability of a mutation in a family. One of the most pressing debates is the addition of multiplex ligation-dependent probe amplification (MLPA) testing to routine mutation detection in BRCA1/2 genes. In order to decide whether our current public testing policy should include BRCA1/2 MLPA for families undertaking BRCA1/2 mutation detection testing, it is essential we ascertain the exact prevalence of MLPA-detected mutations.

**Aim:** To determine the prevalence of large deletions and rearrangements in the BRCA1 and BRCA2 genes in families presenting for genetic testing to the Victorian and Tasmanian Family Cancer Services.

**Method and Results:** As of the 1st August 2007, 1454 families had been tested for BRCA1/2 gene mutations at the six familial cancer clinics in Victoria and Tasmania. Of these, 380 (26.1%) were found to have a mutation with 17 of these (4.5%) detected using MLPA testing and 363 (95.5%) using traditional sequencing. The 1074 families tested and found to be uninformative were selected for BRCA1/2 gene MLPA testing within a study, however 112 were later excluded as their samples could not be tested (82 had a sample that was not suitable for testing, 11 had a duplicate family member tested elsewhere, 2 had gone on to have private testing and 17 samples were unavailable for testing).

Of the remaining 962 families tested using MLPA in this study, 28 (2.9%) were found to carry a large deletion bringing the entire number of families that have a pathogenic mutation to 408. Therefore, of the total 408 families (380 previously identified plus additional 28 identified in study) known to be positive for a BRCA1 and BRCA2 mutation, 45 (11%) have a mutation detected only by MLPA (17 previously identified and 28 identified in study).

**Conclusions**

- MLPA-detected BRCA1/2 mutations are an important proportion (11%) of all BRCA mutations detected.
- The addition of a technique to identify large deletions and gene rearrangements is an essential component of the BRCA mutation detection strategy.
- MLPA, or equivalent, should be included as part of the standard BRCA mutation screen in Victoria and Tasmania.
THE IDENTIFICATION OF CLINICALLY SIGNIFICANT MUTATIONS AND UNCLASSIFIED VARIANTS IN THE BRCA1 AND BRCA2 GENES

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Every year approximately 10 million people are diagnosed with cancer in the world. Breast cancer is one of the four most frequently occurring malignant tumours in humans. Approximately 10% of malignant breast cancers are the result of a genetic predisposition. Contemporary medical technology and diagnostics make early breast cancer detection possible.

Genetic screening of the BRCA1 and BRCA2 genes was implemented at the Institute of Medical and Veterinary Science, South Australia in the year 1996. From 2000-2009, the Division of Genetics and Molecular Pathology, Familial Cancer Section has performed BRCA1/2 genetic screening on 661 patients. A total of 36 and 33 patients have been identified with clinically significant mutations in the BRCA1 and BRCA2 genes, respectively, representing 10.4 % of total cases screened by the laboratory. Furthermore, 28 patients were found to possess unclassified variants (UCV) in the BRCA1 gene, with 58 patients identified with UCV in the BRCA2 gene. A total of 454 presymptomatic tests were performed based on results derived from the genetic screening process. The information that will be addressed during the presentation includes current laboratory testing methodologies and the genotype of detected mutations.
ADHERANCE TO RISK MANAGEMENT RECOMMENDATIONS BY WOMEN WITH LYNCH SYNDROME.

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Lynch syndrome (previously known as Hereditary Non-Polyposis Colorectal Cancer) is caused by a germline mutation in a mismatch repair gene. Patients with Lynch syndrome have a significantly increased risk of developing colorectal cancer and other cancers. Women also have an increased risk of developing endometrial cancer (40% lifetime risk) and ovarian cancer (10% lifetime risk).

Australian screening recommendations for Lynch Syndrome were last published in 2006. Colonoscopy is advised 1-2 yearly. Upper GI endoscopy had been recommended in the past, however in NSW this was revised recently and gastroscopy is now only recommended if there is a family history of gastric cancer. In the past, although total hysterectomy was always offered after childbearing, some gynaecological screening was advised for those who did not proceed with risk reducing surgery. Now such screening would not be routinely advised, given the lack of evidence for its benefit.

The purpose of this study was to obtain accurate data on the uptake of risk-reducing total hysterectomy by women with confirmed Lynch syndrome. Eighty-nine living females (aged between 30 and 70 years) with germline mismatch repair mutations were identified from the Westmead Familial Cancer Service database. Of these, 39 were already known to have undergone a total hysterectomy (either for risk-reduction, cancer treatment or for other medical reasons). The remaining 50 females were contacted via telephone for review and update. Their reasons for delaying or declining risk-reducing surgery were explored. The patients were also updated about the recent change in recommendations for gynaecological cancer screening and upper GI screening. Bowel cancer screening was also documented.

On contact with the selected patients, the following aspects of their care were reviewed:
1. Risk reducing surgery since last appointment
2. Reasons for delaying or declining such surgery
3. Gynaecological screening
4. Current bowel and upper GI cancer screening practice
5. Registration with the Hereditary Cancer Register
6. Changes to family history

While up to 80% of appropriately aged female BRCA1/2 mutation carriers at the Familial Cancer Service elect for risk reducing bilateral salpingo-oophorectomy, the uptake of risk reducing gynaecological surgery in women with Lynch syndrome has not been previously examined. Out of the 50 Lynch syndrome patients to be contacted, 28 have been contactable to date. Seven of these women have undergone total hysterectomy. Overall out of the 67 patients for whom data is currently available, 46 (68.7%) have had a total hysterectomy. The patients’ reasons for having this surgery or for delaying or declining this surgery will also be reported.
#13

**MLH1 WHOLE GENE DELETION WITH EARLY ONSET OVARIAN CANCER AND INTELLECTUAL DELAY – INHERITED OR DE NOVO?**

Jessica Koehler, Prince of Wales Hereditary Cancer Clinic

Case Report: In May 2008, a 33 year old woman (MJ) was referred to the Hereditary Cancer Clinic following her diagnosis of endometroid ovarian cancer. She came from a large family with no known colorectal or endometrial cancer. MJ was a mildly intellectually delayed individual with multiple social and family troubles. She had no dysmorphic features.

Subsequent tumour testing and mutation analysis detected a whole gene deletion of MLH1. Karyotype studies were normal and CGH array further characterised the 3.81-3.84Mb deletion involving MLH1 and several other known genes. It is difficult to conclude whether the deletion is the cause of MJ’s intellectual delay. Predictive testing is underway to determine whether the deletion is de novo or inherited.

This case demonstrates the importance of mismatch repair tumour testing in early onset ovarian cancer cases regardless of family history. Interestingly, MJ’s lack of family history and her developmental delay could potentially be explained by a de novo 3p21.3 deletion.
#14
IMMUNOHISTOCHEMISTRY AND MICROSATELLITE INSTABILITY TESTING IN COLORECTAL CANCER – NOT A MISMATCH

S. C. Hicks, N. J. Hawkins, Q. Nguyen and R. L. Ward

Immunohistochemistry (IHC) and microsatellite instability (MSI) testing are two complementary tests for the detection of Mismatch Repair Deficient (MMRD) colorectal cancers. Approximately 15% of these cancers are characterised by MMRD, with enzyme loss occurring sporadically in over 12% of cases, while in the remaining 2-3% of cases the loss is caused by hereditary non-polyposis colorectal cancer (HNPCC).

For the past two years, our group has promoted the use of MMRD testing on all colorectal cancer cases (approximately 850 cases per year) diagnosed by the six pathology laboratories servicing all hospitals within the South East Sydney and Illawarra Area Health Service. Cases showing loss of expression of any of the MMR enzymes by IHC (n=238) were sent to our laboratory for confirmatory MSI testing. We found that typical patterns of loss identified by IHC (i.e. paired enzyme loss or three or four enzyme loss) were very specific for MSI, while atypical patterns of loss were more problematic in their interpretation.

Over 50 possible cases of HNPCC were identified from the study participants on the basis of either MSH2/MSH6 loss or MLH1 loss in the presence of wildtype BRAF. The process by which these cases were referred (or not referred) to a Family Cancer Clinic is currently being analysed as part of this study. Interestingly, the subset of 29 MLH1 negative, BRAF wildtype cancers in this group showed a bimodal age distribution, suggesting that the large majority of the 20 cases over the age of 60 years were likely to be sporadic in nature.
#15
VARIABLE MLH1 IMMUNOHISTOCHEMICAL STAINING IN FAMILIES WITH MLH1 GERMLINE MUTATIONS

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Background: Lynch Syndrome (HNPCC) is an autosomal dominant condition which predisposes to early-onset cancer of the colorectum, endometrium, and to a lesser extent, stomach, ureter and renal pelvis, ovary, brain and small bowel. The underlying defect in Lynch syndrome is a germline mutation in one of four DNA mismatch repair (MMR) genes, MLH1, MSH2, MSH6 and PMS2. MLH1 germline mutations usually result in a tumour phenotype demonstrating loss of both MLH1 and PMS2 nuclear staining by Immunohistochemistry (IHC) accompanied by microsatellite instability (MSI). The aim of this study was to identify the frequency of variable MLH1 expression in families harbouring a MLH1 germline mutation and investigate the types of mutations as a potential source of this variable expression.

Design: Tumours (colonic and extra-colonic cancers and colorectal adenomas) from population- and clinic-based probands recruited to the Australasian Colorectal Cancer Family Registry (ACCFR) were characterized for MSI and MMR protein loss by IHC. Germline mutation testing (sequencing and MLPA) was performed in individuals demonstrating MSI from the population-based probands, and for all the clinic-based probands with the gene tested guided by the MMR IHC protein loss. Tumour DNA was also tested for BRAF V600E somatic mutation. Pedigrees were reviewed to assess whether families met modified Amsterdam criteria (mAC).

Results: Seventy-three families enrolled in the ACCFR were found to harbor germline mutations in the MLH1 gene, of which, nine families (9/73, 12%) contained individuals (13 males and 12 females) demonstrating variable loss of MLH1 protein in tumours on IHC (accompanied by consistent loss of PMS2). The average total number of lesions tested per family was 5 (median = 4). The mutation types within these 9 families included 4 affecting splice sites, 2 missense, 2 frameshift and 1 inframe exon deletion. When compared to all ACCFR MLH1 mutation types, splice site mutations and missense mutations were more common in the families with variable MLH1 expression although neither result was statistically significant (p value=0.27, OR=2.21, 95%CI=0.57-8.63 and p value=0.26, OR=2.76, 95%CI=0.54-14.82, respectively). Seven of the nine families met mAC. None of the tumours tested showed the V600E BRAF mutation.

Conclusion: This study demonstrates both intra-individual and intra-family heterogeneity of MLH1 protein expression in Lynch syndrome tumours due to mutations in MLH1. This study highlights the need to consider MLH1 mutations, particularly splice site and missense mutations, when interpreting IHC showing loss of PMS2 protein only. Further investigation is warranted, however, in a larger sample set.
ANALYSIS OF FAMILIES WITH LYNCH SYNDROME COMPLICATED BY ADVANCED SERRATED NEOPLASIA: THE IMPORTANCE OF PATHOLOGY REVIEW AND PEDIGREE ANALYSIS

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The identification of Lynch syndrome has been greatly assisted by the advent of tumour immunohistochemistry (IHC) for mismatch repair (MMR) proteins, and by the recognition of the role of acquired somatic BRAF mutation in sporadic MMR-deficient colorectal cancer (CRC). However, somatic BRAF mutation may also be present in the tumours in families with a predisposition to develop serrated polyps in the colorectum. In a subgroup of affected members in these families, CRCs emerge which demonstrate clear evidence of MMR deficiency with absent MLH1 staining and high-level microsatellite instability (MSI). This may result in these families being erroneously classified as Lynch syndrome, or conversely, an individual is considered “sporadic” due to the presence of a somatic BRAF mutation in a tumour. We present two Lynch syndrome families who demonstrated several such inconsistencies. In one family, IHC deficiency of either MSH2 or MLH1 was demonstrated in tumours from different affected family members, presenting a confusing diagnostic picture. In the second family, MLH1 loss was observed in the lesions of both MLH1 mutation carriers and those who showed normal MLH1 germline sequence. Both families had Lynch syndrome complicated by an independently segregating serrated neoplasia phenotype, suggesting that in families such as these, tumour and germline studies of several key members, rather than of a single proband, are indicated to clarify the spectrum of risk. This study raises the possibility that in “mixed lineage” families, use of BRAF testing to exclude Lynch Syndrome may be misleading.
#17

WE ARE ON THE MANCHESTER BANDWAGON – SO HOW ARE WE DOING?

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**Background:** In September 2007 our unit began prospectively triaging familial breast and/or ovarian cancer families for BRCA1/2 genetic testing using a combined Manchester Score.\(^1\),\(^2\)
This method was chosen because of its simplicity—the score can be calculated manually without need for a computer and the score can be recalculated easily in an outpatient setting following clarification of a family history.

**Aim and Methods:** To review our mutation detection rate using a combined Manchester Score.

**Study population:** A total of 939 probands have had complete BRCA1 and BRCA2 genetic testing by our service and have a combined Manchester Score available; 827 were triaged to testing using the clinical "Adelaide criteria" and had a retrospective combined Manchester score calculated; 112 were triaged to testing using a prospectively calculated combined Manchester score.

**Results:** Pathogenic BRCA1/2 mutations were detected in 152/939 probands tested (16.1%).
A third of probands with a combined Manchester Score ≥20 had a pathogenic BRCA1/2 mutation detected (127/377; 33.6%). The 10% mutation detection threshold was reached at a combined Manchester Score above 15 (see graph; polynomial trendline fitted with \(R^2=0.92\)).

**Conclusion:** Our experience suggests it is appropriate to triage familial breast and/or ovarian cancer families to BRCA1/2 genetic testing when the combined Manchester Score is more than 15.

THE AUSTRALASIAN COLORECTAL CANCER FAMILY REGISTRY (ACCFR) BIOSPECIMEN REPOSITORY

Erika Pavluk\textsuperscript{1}, Daniel D Buchanan\textsuperscript{1}, Michael D Walsh\textsuperscript{1}, Rhiannon J Walters\textsuperscript{1}, Judi Maskiell\textsuperscript{2}, Mark A Jenkins\textsuperscript{2}, Joanne P Young\textsuperscript{1}, Jeremy R Jass\textsuperscript{3}, and John L Hopper\textsuperscript{2} on behalf of the Australasian Colorectal Cancer Family Registry

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The Australasian Colorectal Cancer Family Registry (ACCFR) is one of six international sites that make up the Colorectal Cancer Family Registry (Colon-CFR). This consortium provides a comprehensive infrastructure for facilitating interdisciplinary studies in the genetic epidemiology of colorectal cancer. It is the goal of the CFR to become a world leading resource of data and biospecimens for elucidating the etiology of colorectal cancer, and for developing effective means of prevention and mortality reduction. The specific aims of the ACCFR include: 1) Data collection of medical history, environmental exposures, family history and also biospecimens from affected individuals through both population-based and ‘high-risk’ clinic ascertainment throughout Australia and New Zealand; 2) Conducting active and passive follow-up of consented cases and their family members including clinical follow-up; 3) Performing molecular characterization, including testing for microsatellite instability (MSI), mismatch repair (MMR) IHC, methylation of the \textit{MLH1} promoter, somatic \textit{BRAF} V600E mutation and germline mutation testing in the \textit{MLH1}, \textit{MSH2}, \textit{MSH6}, \textit{PMS2} and \textit{MYH} genes of selected Colon CFR participants; 4) Adding to and maintaining the biospecimen repository comprised of blood samples and tumour sections and 5) Participating in the Colon-CFR and external CFR collaborations.

The ACCFR is now in its third phase of funding and recruitment, and has accumulated a substantial biorepository of blood and tissue samples, many of which have received extensive molecular characterization and pathology review. Population-based ascertainment has recruited in excess of 800 CRC-affected probands all with a blood draw. Additionally, CRCs have been collected, characterised for MSI, MMR IHC and the \textit{BRAF} V600E mutation for approximately 95% of these population-based probands. Clinic-based recruitment has identified over 500 families with multiple affected family members. On average, blood samples are available from 8 affected and unaffected members per family. Multiple tumours, including CRCs, extra-colonic cancers and gastrointestinal polyps have been collected for each family. Screening for Lynch Syndrome (HNPCC) has identified in excess of 730 MMR mutation carriers from 204 families from all study phases (73 \textit{MLH1}, 100 \textit{MSH2}, 18 \textit{MSH6} and 13 \textit{PMS2} mutation families).

The ACCFR is actively looking to establish collaborations with researchers who are interested in conducting research that will utilise this resource.
CAUSATIVE MUTATIONS IN LYNCH SYNDROME: WHAT ARE WE MISSING?

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The current practice for identifying individuals with Lynch syndrome (defined as carriers of a deleterious mutation within one of the four primary DNA mismatch repair (MMR) genes, MLH1, MSH2, PMS2 or MSH6) involves obtaining an extensive family history and assessing Lynch syndrome associated tumours for microsatellite instability and/or immunohistochemical (IHC) loss of MMR proteins. Subsequent mutation screening for large duplications/deletions via MLPA, and exonic sequencing are guided by IHC results. Serrated neoplasia families are separated from Lynch syndrome via somatic BRAF mutation testing in MLH1 deficient tumours. This approach has proven both logical and efficient for the identification of mutations in the MMR genes, however there are still many families who fail to have a mutation identified, despite multiple affected individuals displaying loss of staining for one or more of the MMR proteins. The inability to identify a mutation in these families is of significant clinical importance as individuals, who have an increased risk of developing CRC, cannot be identified.

In an attempt to address these issues we performed basic linkage analysis, across three regions that contain the four main MMR genes (2p21, 3p22.2 and 7p22.1), in families where a mutation had not been identified despite immunohistochemical indications of gene inactivation in these regions. Three families containing a known mutation were used as a proof of principle, and in each case a common, mutation associated, haplotype was identified that was not present in any non-mutation carriers. Within each family where no mutations had been detected, we were able to identify a common haplotype which correlated with the IHC findings of affected individuals. These haplotypes suggest that any causative mutation in these families lies in cis with the IHC-associated gene, and as such, current screening procedures must be failing to detect them. Regulatory regions such as promoters and intronic enhancer elements may prove to be a source of mutations that affect transcription and/or translation, and the recent identification of germline methylation of MSH2 and mutations in the TACSTD1 gene also supports a role for mutation screening beyond current practices. Such common haplotypes may also prove to be very useful from a clinical point of view, as they could be used as an alternative way to identify family members who are at an increased risk of developing Lynch syndrome.
#20
CLASSIFYING MISMATCH REPAIR GENE VARIANTS IDENTIFIED IN COLON CANCER PATIENTS USING A COMPREHENSIVE APPROACH

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A considerable proportion of Lynch syndrome families present with mismatch repair (MMR) gene sequence variants of uncertain clinical significance, which constitute a challenge in both the research and clinical settings. MMR gene unclassified variants (UVs) may result in aberrations (splicing, mRNA instability or protein instability or altered function), and thus be associated with increased cancer risk. UVs include rare nucleotide changes predicted to cause missense substitutions, predicted to cause possible alterations in splicing, and small in-frame deletions. We are investigating the pathogenicity of 50 UVs from Australian families enrolled in the Colon Cancer Family Registry (Colon CFR). A literature search aided by CHIP SNPper and the Leiden Open Variation Database (LOVD) was done to exclude polymorphic variants, and to clarify variant pathogenicity based on published evidence. The search excluded 8 variants as common polymorphisms (frequency ≥ 1%), identified 5 variants as clinically significant, and 13 variants were shown to be novel. Bioinformatic programs were used to predict the effect of the gene variants on pre-mRNA splicing. The combined evolutionary conservation and physicochemical properties of amino acid alterations was also examined for the MLH1 and MSH2 variants. We are currently assessing remaining UVs using a comprehensive approach. This includes control screening, in vitro splicing assays, and assessment of tumour pathology (IHC, MSI) and clinical data from relevant families. Australian controls have been genotyped using the iPlex Sequenom MassArray® system, including a method to avoid PMS2 pseudogene amplification. We are screening cycloheximide-treated variant carrier lymphoblastoid cell lines for splicing aberrations. Family genotyping and tumour pathology assessment are in progress. In addition, we are conducting allele-specific quantitative real-time PCR to identify the relative contributions of missense and splicing aberrations to the function of the protein encoded by MLH1 c.113A>G, identified in a previous study. Results from ongoing research will be presented.
#21
IDENTIFYING LESIONS OF IMPORTANCE IN COLONIC SERRATED POLYPOSIS

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Background: Serrated polyposis syndrome (SPS) is a rare syndrome characterized by development of numerous colorectal serrated polyps, and is associated with an increased risk of colorectal cancer (CRC). The sessile serrated adenoma (SSA) has been recognized in recent years as a potential precursor to some CRCs. However, accurate, consistent diagnosis of SSAs continues to be elusive, with poor reproducibility in diagnosis even amongst experienced pathologists. In a recent study, Owens and colleagues reported expression of gastric mucin MUC6 in SSAs but not conventional hyperplastic polyps (HPs) suggesting diagnostic utility for this marker. We have previously reported an association between altered mucin expression and microsatellite instability in CRCs mirroring patterns observed in serrated polyps, and were intrigued by the possible involvement of another mucin in serrated neoplasia.

We aimed to assess expression of MUC6 gastric mucin in a series of colorectal polyps and CRCs from patients with SPS, to relate expression to clinicopathological features and expression of the DNA mismatch repair proteins MLH1 and PMS2.

Design: Tissue samples were available from 82 individuals who met the WHO criteria for SPS. The study cohort comprised 50 females and 32 males (mean age 52 years) providing a total of 349 polyps and 23 CRCs for investigation. Sections of each lesion were stained for MUC6, MLH1 and PMS2. DNA was also extracted for assessment of somatic KRAS and V600E BRAF mutations. All histological material was subjected to full histopathological reassessment.

Results: Overall, MUC6 expression was detected in 51% polyps and 39% CRCs. Within polyps, MUC6 was restricted to the crypt bases, whereas expression within CRCs showed considerable heterogeneity. MUC6 expression was associated with the presence of somatic BRAF mutation and also loss of MLH1 and PMS2 in CRCs and polyps. Larger and more proximal polyps were most likely to express MUC6. Serrated polyps of all types were more often MUC6-positive than traditional adenomas, and SSAs were more often positive than conventional HPs (80% vs. 48%).

Conclusions: MUC6 is expressed by a significant proportion of SSAs as well as approximately half of HPs in SPS thus limiting its value as a discriminating marker. MUC6 expression is associated with proximal location, somatic V600E BRAF mutation, and loss of mismatch repair proteins MLH1 and PMS2. This study also highlights the increased risk of CRC in patients with SPS, with 23 CRCs occurring in 22/82 (27%) of participants.
DETERMINANTS OF ACQUIRED CHEMO-RESISTANCE IN OVARIAN CANCER

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Ovarian cancer is the 5th most common cancer in women in Western countries, and the most lethal gynaecologic malignancy. Despite aggressive surgery and multi-drug chemotherapy the majority of women experience disease recurrence and ~70% of women will succumb to the disease. Previous studies from our laboratory have identified key mechanisms associated with primary resistance in advanced-stage serous cancers by screening for somatic copy number changes (CNC). However, the molecular changes that occur in serous ovarian tumours during the development of acquired resistance are poorly understood.

We therefore aim to identify key mechanisms associated with acquired chemo-resistance in serous ovarian cancers utilising an integrated genome-wide analysis of DNA CNC and gene expression (GE). These studies explicitly involve the comparison of ovarian tumour tissue collected at the time of primary surgery with patient-matched ascites samples to increase the power of the analysis. Unique regions of copy number gain or loss within individual sample pairs, and across the cohort, are correlated with changes in GE to identify candidate genes associated with acquired chemo-resistance. Samples are drawn from a clinically well-defined subset of the Australian Ovarian Cancer Study (AOCS) cohort and include patients with optimal debulking (<1cm residual disease) and who were treated with a platinum based agent following surgery. Tumour cells were isolated from ascites collected at relapse.

Preliminary analysis of CNC in 11 patient-matched primary tumour and relapse ascites has identified 2 regions of copy number gain, at 3q24-26.3 and 8q24.13-8q24.21, common in 55% of ascites. Additionally, 2 regions of copy number loss, at 9p23 and Xq21.31 - 21.33 have been identified as unique in 55% of ascites. Copy number gains on 3q and 8q have been noted previously in primary ovarian tumours and are usually attributed to gain of EVI1/MDS1 and MYC respectively. Our observations suggest that increasingly high-level amplification at these loci is common in relapse samples. Further, a deletion containing a novel candidate gene has been identified at 9q23: PTPRD (protein tyrosine phosphatase delta), observed to be lost or mutated in several other malignancies and is associated with poor prognosis in breast and colon cancer. We are currently undertaking mutation detection of PTPRD in relapse ascites by High Resolution Melt (HRM) to determine whether the remaining allele is mutated.
GENETIC VARIANTS ASSOCIATED WITH RESPONSE TO PLATIN-BASED CHEMOTHERAPY IN INVASIVE OVARIAN CANCER

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Carboplatin is commonly used in treating head and neck, lung and gynaecological cancers. However, its utility is limited because some patients develop severe treatment-induced toxicity while some do not respond to treatment. We hypothesized that genetic factors are likely to contribute to variation in response to, and toxicity caused by, chemotherapeutic agents. In an attempt to elucidate such factors, we applied a genome-wide approach integrating genotypes, mRNA expression and cellular sensitivity to carboplatin using HapMap lymphoblastoid cell lines (LCLs). The concentration of carboplatin required to inhibit cell growth was determined, and the percentage of surviving cells at different treatment concentrations along with carboplatin IC\textsubscript{50} (concentration required to inhibit 50\% cell growth) were used as carboplatin sensitivity phenotypes. Genome-wide mRNA expression was determined using the Affymetrix GeneChip\textsuperscript{®} Human Exon 1.0 ST Array and genome-wide association studies were performed to identify genetic variants significantly associated with carboplatin sensitivity through their effects on mRNA expression. We identified 65 SNPs that were associated with mRNA expression and at least one carboplatin sensitivity phenotype, five of which were replicated in a separate set of LCLs. We analyzed these five SNPs in 543 women with invasive ovarian tumours recruited by the AOCS, and treated with a minimum of 4 cycles of paclitaxel (135 or 175 mg/m\textsuperscript{2}) and carboplatin (AUC 5 or 6). One SNP (rs16499942) was significantly associated with progression free survival (adj. HR\textsubscript{per-allele} 1.24 (1.02 – 1.49) p=0.03) in all cases, with a more pronounced effect in a subset of women with optimally debulked tumours (residual disease ≤ 1 cm) (adj. HR\textsubscript{per-allele} 1.39 (1.09 – 1.76) p=0.007). This SNP is located in \textit{NRG3} gene and is significantly associated with the expression of several other genes. These findings support the utility of cell-based models and genome-wide approaches to the identification of SNPs that contribute to variation in chemotherapeutic response and toxicity, and subsequent validation clinical studies.
HIGH RESOLUTION MELT ANALYSIS OF SOMATIC KRAS MUTATIONS IN ENDOMETRIAL CANCERS

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Background. Approximately 30% of colorectal cancers (CRCs) and 10-30% of endometrial cancers (EC) present with somatic mutations in codons 12 and 13 of the KRAS gene. CRCs with KRAS mutations have reduced sensitivity to specific chemotherapy, supporting the clinical screening for KRAS mutations. The role of somatic KRAS mutations in EC is less clear. Historically, KRAS mutation detection has involved direct sequencing, a time consuming, costly and insensitive when the percent of tumour within a sample is low. High resolution melt (HRM) analysis is a sensitive, rapid screening tool for detecting sequence variation. The aim of this study was to explore HRM analysis as an inexpensive screening assay for KRAS mutations in EC. Methods. EC from 40 individuals (test set) were identified via the Victorian Cancer Registry from individuals recruited as part of the Melbourne Collaborative Cohort Study. A second sample set (study set) of 188 patients with young onset <50yrs EC were identified from the Queensland Centre for Gynaecological Cancer. DNA was extracted from micro-dissected formalin-fixed paraffin embedded (FFPE) tumour sections and assayed for KRAS mutations (codons 12 and 13) using HRM and capillary sequencing. Primers for HRM amplicons of 76bp and 92bp were tested utilising the SYTO9 dye under standard PCR conditions. Results. The pilot sample set of 40 EC cases revealed definitive HRM results for 31/40 (78%) EC cases, with the smaller 76bp product amplifying more samples successfully when compared to the 92bp HRM amplicon, 78% (31/40) versus 55% (22/40), respectively. Of the 31 EC cases that amplified, 8 (25.8%) showed a aberrant HRM melt profiles. Direct bi-directional sequencing analysis confirmed the presence of KRAS mutations in 6/8 (75%) samples showing aberrant HRM profiles, with 2 samples (2/8, 25%) discordant between HRM and sequencing, suggesting either increased sensitivity of the HRM assay over sequencing or false positive HRM results. The 188 young-onset ECs (<50yrs) were screened by HRM analysis using the 76bp amplicon with 74.5% (140/188) generating normal KRAS HRM melting profiles and 24 (12.8%) demonstrating an abnormal melt profile, with all 24 samples confirmed as somatic mutations by sequencing. Conclusion. The 76bp HRM amplicon successfully amplified 85% of the FFPE EC samples assayed with excellent concordance with sequencing in identifying KRAS positive samples highlighting this technique as a sensitive and cost-effective alternative to direct sequencing for identifying KRAS mutations in EC.
#25
THE AUSTRALIAN NATIONAL ENDOMETRIAL CANCER STUDY (ANECS) - RESULTS FROM HIGH-RISK AND LOW-RISK GENETIC STUDIES
Kaltin Ferguson, Tracy O’Mara, Bryony Thompson, Carol Paterson, Felicity Lose, Michael Walsh, Sven Arnold, Dan Buchanan, Joanne Young, Penelope Webb, Amanda Spurdle, on behalf of the Australian National Endometrial Cancer Study
Queensland Institute of Medical Research and Montserrat Garcia-Closas, Louise Brinton, Jim Lacey, Mia Gaudet, Hannah Yang, on behalf of PECS (Polish Endometrial Cancer Study), National Institutes of Health, National Cancer Institute, USA
Douglas Easton, Alison Dunning, Paul Pharoah, Katie Gregory, Shahana Ahmed, on behalf of SEARCH (Studies of Epidemiology & Risk factors in Cancer Heredity), Cambridge University, UK

The Australian National Endometrial Cancer Study was set up to establish an integrated approach to endometrial cancer research. The primary aims of the Study are to: clarify existing and identify new modifiable risk factors for Endometrial Cancer, and their interaction with genetic factors, by subtype; examine the genetic basis to disease within population-based cases and multiple case families; establish and maintain a biorepository and epidemiological, molecular and clinical database for ongoing studies. In collaboration with a network of more than 40 clinicians, the study has recruited 1496 women with endometrial cancer, 746 population controls identified via the electoral roll, 828 controls identified via the Australian Red Cross Blood Services, and 236 relatives of cases reporting a family history of cancer. Participants provided information on risk factors, dietary habits, a blood sample if possible, and clinical data if relevant. Fresh and/or archival tumour samples were collected for a subset of cases and tissue microarrays have been constructed where material was available. We are conducting ongoing screening of cases for tumour features suggestive of mismatch repair (MMR) gene mutations. Immunohistochemical analysis of 476 unselected cases has identified 167 (26%) with loss of expression of mismatch repair (MMR) protein(s). Mutation screening to date has identified MMR gene mutations for 3% of cases, with MSH2 and/or MSH6 loss suggesting positive mutation status for another 4%. We have also used high-throughput Sequenom iplex genotyping to investigate 57 candidate SNPs, in collaboration with international researchers. Candidate SNPs were chosen via three approaches: those identified through an endometrial cancer literature review; those identified from association studies of other cancers; and SNPs from genes shown to have least variable expression in lymphoblastoid cell lines (see presentation by Lose et al). Genotyping was carried out on all ANECS cases and controls with available DNA, as well as cases and controls from PECS and SEARCH for selected SNPs. Of the 57 SNPs genotyped, 3 were found to be associated with risk: ESR1 rs3020314 (per allele OR 1.13 (1.02-1.25); P= 0.03); TCF2 rs4430796 (per allele OR 0.87 (0.77-0.97); P=0.009); CYP19A1 rs700519 (per allele OR 0.60 (0.38-0.92); P=0.02). Replication studies for the ESR and TCF2 SNPs are in progress. The ESR SNP was previously reported to be associated with increased breast cancer risk, while the TCF2 SNP was identified by multiple genome-wide association studies to be associated with increased prostate cancer risk and decreased risk of diabetes. Results from analyses to date will be presented.
VARIATION IN TWO INNOVATIVE CANDIDATE GENES, *ABAT* AND *NELL1*, AND CANCER

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It is well known that genetic variation in or near genes can cause changes in expression and lead to cancer initiation or progression. A study of baseline gene expression in lymphoblastoid cell lines from healthy individuals (Cheung et al. Nat Genet 2003) reported that the expression levels of some genes varied greatly between individuals, whereas the inter-individual variation in expression of other genes was minimal. We reasoned that those genes that display little expression variability are likely to be involved in vital processes that are tightly controlled to maintain normal cell function and behaviour. Hence, single nucleotide polymorphisms (SNPs) in these genes may alter gene expression and could lead to cancer.

Cheung et al. kindly provided us with a list of 100 genes that showed the least variability in gene expression, termed “least variable genes”. Interestingly, the large majority of these genes were involved in one of two essential and highly controlled cellular processes often disrupted in cancer: cell growth control (proliferation and apoptosis) and signal transduction. A Pubmed search also revealed that 96% of these genes were aberrantly expressed in cancers and/or located in chromosomal regions associated with cancer.

To focus our approach, we searched the Cancer Genetics Markers of Susceptibility (CGEMS) data to determine if any of the 100 least variable genes were associated with cancer risk, and found that multiple polymorphisms were associated with both breast and prostate cancer risk (P≤0.01) in two of these genes, *ABAT* and *NELL1*. Intriguingly, all of these SNPs in each of the genes are associated with either breast or prostate cancer, not both. This is reminiscent of the 8q24 multiple cancer-linked region, where numerous cancers (such as breast, prostate, colorectal and ovarian cancers) are associated with various SNPs in a 1.18Mb region on 8q24, although each cancer is not necessarily associated with the same 8q24 SNP/s as another cancer.

We genotyped *ABAT* and *NELL1* SNPs reported to be associated with cancer in the CGEMS study in prostate cancer cases (n=944) and male controls (n=1354) and endometrial cancer cases (n=1318) and female controls (n=1164) using the Sequenom MassARRAY genotyping platform. No association was detected between any of the *NELL1* SNPs and endometrial cancer or prostate cancer and none of the *ABAT* SNPs were associated with endometrial cancer in our sample set. Preliminary analysis revealed that all three of the CGEMS prostate cancer-associated *ABAT* SNPs were associated, or borderline associated, with prostate cancer in our study. Results of further analyses, including results from a replication set supplied by Eeles et al., will be presented.
GENETIC VARIANTS, PHENOTYPIC SPECTRUM AND BREAST CANCER RISK ASSOCIATED WITH GERMLINE MUTATIONS OF \textit{PALB2}: IDENTIFYING FEMALE \textit{PALB2} CARRIERS AT THE TIME OF DIAGNOSIS

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A family history of breast cancer is one of the most important risk factors for the disease. Having a first-degree relative with breast cancer is associated with, on average, doubling a woman’s risk of the disease. Over 90% of breast cancer cases occur in women who are at above median familial risk \(^1\). To date, population studies of female breast cancer show only a small proportion of familial aspects of breast cancer can be explained by current knowledge of its causes. Finding all causes of familial aggregation will lead to a major increase in understanding of the causes of breast cancer per se. \textit{PALB2} was discovered as a protein that associates with \textit{BRCA2} \(^2\) and recently with \textit{BRCA1}. \textit{PALB2} co-localizes with \textit{BRCA1} and \textit{BRCA2} in nuclear foci\(^3\). It has been suggested by Sy et al., 2009 that \textit{PALB2} is involved in \textit{BRCA1} and \textit{BRCA2} binding in a complex that is concentrated at DNA breaks which has been found to be involved in homologous recombination repair \(^3\). Mutations in \textit{PALB2} have been reported to be associated with an average of two to three fold increased risk of breast cancer. It is predicted that \textit{PALB2} mutations are associated with ‘high risk’ when detected in the context of strong family history.

The aim of this project is to identify women with \textit{PALB2} mutations by conducting a large case-control family study. We will screen the earliest onset breast cancer cases from families recruited from genetics population-based cases to characterize \textit{PALB2} mutations in terms of breast cancer risk. After which, criteria will be devised to identify, at time of diagnosis, the women most likely to carry pathogenic \textit{PALB2} mutations. Using High Resolution Melt (HRM) curve analysis and Sanger sequencing, we have screened, to date, 756 early on-set population-based cases and 400 women from multiple-case breast cancer families. From the population-based families, we have identified 2 carriers of \textit{PALB2} mutations with extremely strong family histories of breast cancer. Another 4 were identified as carriers of \textit{PALB2} mutations from the clinic-based cases. Testing for mutations in relatives of the carriers has identified an additional 20 \textit{PALB2} mutation carriers.

\textit{PALB2} mutations are rare, but the implications of carrying a mutation in \textit{PALB2} are high and could potentially influence treatment choices. More work needs to be done on characterizing these mutations and identify female carriers to provide them with optimal evidence based models of ongoing surveillance and care.

#28
RAPID SCREENING OF BRCA1 AND BRCA2 MUTATIONS USING HIGH RESOLUTION MELTING (HRM).

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There is a pressing need for methodologies that can reduce the cost of genetic testing for inherited predisposition to cancer. High Resolution Melting (HRM) is a technique that is used to detect sequence variations within an amplified region of DNA. This is achieved using a fluorescent dye which specifically intercalates with double stranded DNA. Monitoring the fluorescence of saturating concentrations of the dye as the temperature is increased allows us to monitor the sequence specific denaturation (melting) of the amplicon. By comparing the melting behaviour of patient samples to a wildtype control, it is possible to determine whether a sample contains sequence variations which may in turn be identified via sequencing.

We have developed the technique to screen DNA samples for germline and somatic mutations within the breast cancer predisposition genes BRCA1 and BRCA2. The assay consists of 86 amplicons covering the coding region and intron-exon boundaries of BRCA1 (33) and BRCA2 (53). We tested each amplicon with wildtype controls and positive mutation controls to ensure that the amplicons used could detect all mutations present. 189/190 different mutations were detectable in BRCA1 and 272/274 different mutations were detectable in BRCA2.

Samples can either be screened one amplicon at a time, or more commonly, have all amplicons screened at the same time. The latter is being used to evaluate HRM as a routine diagnostic method. To date, 128 unknown samples have been screened and 13 pathogenic mutations have been detected. Common polymorphisms are also detected by HRM. Nevertheless, the number of amplicons requiring sequencing can be reduced to between 5-10 amplicons following HRM screening. We are also beginning to screen ovarian cancer samples for somatic mutations in the BRCA1 and BRCA2 genes.
#29  
THE APPLICATION OF SNP-BASED ARRAY CGH FOR THE DETECTION OF NOVEL CANCER SUSCEPTIBILITY GENES IN MULTI-CANCER FAMILIES  

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Most recognised heritable cancer syndromes, apart from Li Fraumeni Syndrome which is caused by mutations in TP53, have a restricted range of tumour types associated with them, even though most of the responsible tumour suppressor genes are ubiquitously expressed. We hypothesise that there are additional, generic, cancer susceptibility syndromes, caused by rare germline mutations in other tumour suppressor genes, and that loss of heterozygosity (LOH) will constitute the ‘second hit’ in these tumours. There are seldom enough living affected individuals in these ‘general cancer families’ for linkage analysis because of the high mortality rates of many cancers, but we propose that the genes responsible can still be located by SNP-based array CGH analysis, because all the tumours in the family will share retention of the risk haplotype but lose the other allele.  

We identified six non-BRCA1/2 families ascertained by kConFab with a significant excess of multiple cancer types, as well as two non-CDKN2A families with multiple cases of melanoma and other cancer types. Because the tumours in these families are often small and the DNA limited, we assessed the performance of formalin-fixed, paraffin embedded (FFPE) tumour samples on the GoldenGate Assay (Illumina) using small amounts of template DNA. We found that lower amounts of DNA input (down to 75ng) perform adequately in the GoldenGate assay, and that loss of information and miscalling was minimal when SNP calls for the FFPE samples were compared to matched fresh frozen pairs. In addition, SNP-CGH profiles were very similar across replicate samples of differing DNA amounts and the matched frozen control tumour. We also assessed the performance of a panel of 30 FFPE tumours from the multiple cancer families we had identified, that ranged in tumour site, age of pathology block (2-20 years) and processing pathology lab. Included in this panel were tumours of the breast, ovary, colon, testis, pancreas, brain, stomach, thyroid and melanoma. We obtained SNP genotyping data from 28/30 tumours and used the R package beadarraySNP to perform analysis of DNA copy number and LOH on these tumours, comparing tumours within each family to identify common regions of loss or gain. We detected large regions of loss and gain, and also screened smaller, focused regions around known cancer genes for aberrations. This approach will be combined with genetic linkage analysis in families where germline DNA samples are available to identify candidate cancer predisposition genes in families with apparent inherited cancer susceptibility but for which no known cancer syndrome is apparently responsible.
#30
**BRCA2 SEQUENCE VARIANTS CAUSING MISSENSE ALTERATIONS AND SPLICING ABERRATIONS: IMPLICATIONS FOR PREDICTION OF PATHOGENICITY**

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Missense substitutions in high-risk cancer susceptibility genes create clinical uncertainty in the genetic counseling process. Multifactorial likelihood classification approaches and *in vitro* assays are useful for the classification of exonic sequence variants in *BRCA1* and *BRCA2*, but these rely on the assumption that changes in protein function are the major biological mechanism of pathogenicity *in vivo*. This study investigates the potential role of aberrant splicing in pathogenicity for exonic variants. A panel of five exonic BRCA2 variants predicted to encode missense substitutions were analysed using patient RNA to investigate the role of aberrant splicing in variant pathogenicity. Centrosome amplification and homologous recombination assays were utilized to investigate protein function. RT-PCR analysis identified splicing aberrations for c.7988A>T (p.Glu2663Val) and c.8168A>G (p.Asp2723Gly). However, both variant and wildtype alleles were also present in full-length mRNA transcripts, suggesting that both missense and wildtype proteins would be produced by variant carriers. Assays of protein function indicate that the p.Glu2663Val, p.Asp2723Gly and p.Arg3052Trp missense proteins have abrogated function consistent with predicted pathogenicity. No splicing aberrations were identified for c.7336A>G, c.8839G>A, and c.9154C>T. Multifactorial likelihood analysis provided evidence for pathogenicity for c.7988A>T (p.Glu2663Val), c.8168A>G (p.Asp2723Gly) and c.9154C>T (p.Arg3052Trp) supporting evidence derived from experimental analysis. The observation that a subset of exonic sequence variants may cause splicing aberrations and missense alterations has implications for the application of prior probabilities in multifactorial likelihood methods used to predict variant pathogenicity. Possible splicing aberrations should not be discounted when evaluating the clinical significance of exonic variants.
DO MUTATIONS IN PALB2 CAUSE MALE BREAST CANCER?

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The identification of mutations in \textit{PALB2} (Partner And Localizer BRCA2) is very important for some families that have multiple cases of female breast cancer. Recent observations of male breast cancer occurring in families with \textit{PALB2} mutations suggest that \textit{PALB2} may also play a role in the male breast cancer predisposition. To explore this possibility we screened germline DNA from 25 men diagnosed with breast cancer participating in the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab).

To screen these DNAs for mutations in \textit{PALB2} we applied High Resolution Melting (HRM) curve analysis and DNA sequencing technology. HRM analysis is a new technology which allows the amplification of DNA by PCR using fluorescent dyes that bind specifically to double-stranded DNA. When bound to DNA they fluoresce brightly but in the absence of double-stranded DNA, they only fluoresce at a low level. A genetic variation in the DNA region amplified will alter the temperature at which the DNA strands melt apart and so the melt curve will appear different. The difference may only be very minor but because the HRM machine has the ability to monitor this process in “high resolution”, it is possible to accurately document these changes and therefore identify DNA fragments that may contain genetic variation.

The HRM process is extremely cost effective and rapid when compared to other mutation detection methods.

We will present the outcome of the analysis of \textit{PALB2} in the 25 men affected with breast cancer from multiple breast cancer families.
#32
INHIBITING NONSENSE-MEDIATED MRNA DECAY FOR BREAST CANCER GENE IDENTIFICATION

J. Johnson¹,², N. Waddell¹, K.K. Khanna¹, M. Brown², kConFab and G. Chenevix-Trench³
¹Queensland Institute of Medical Research, ²University of Queensland

Transcripts containing premature termination codons (PTCs) are selectively and rapidly degraded by the nonsense mediated mRNA decay (NMD) pathway. Noensie and Dietz (2001) proposed a strategy for disease gene identification by pharmacologically inhibiting the NMD pathway to stabilise these mutant transcripts. This technique is known as GINI (Gene Identification by Nonsense-mediated mRNA decay Inhibition). GINI has proved successful in identifying mutations in colorectal, prostate and breast cancer cell lines. However, the approach has not yet been applied to identify germline mutations.

Roughly 5-10% of breast cancer cases are attributable to an inherited predisposition. However, only about 30% of all familial breast cancer cases can be explained by the known moderate- and high-risk breast cancer susceptibility genes (BRCA1, BRCA2, STK11, TP53, PTEN, ATM, CHEK2, PALB2, and BRIP1). It is likely that rare mutations exist in novel breast cancer genes that explain some of the multiple-case families. We have therefore applied the GINI technique on lymphoblastoid cell lines (LCLs) derived from affected and unaffected members of three non-BRCA1/2 hereditary breast cancer families from kConFab in order to identify candidate susceptibility genes with nonsense codons.

The three non-BRCA1/2 families (A, B and C) had high Manchester scores (19 and above) and thus have a high probability of having a mutation other than BRCA1 or BRCA2. Family A, B and C had a total of eight, seven and nine LCLs derived from individuals, respectively. NMD was inhibited using caffeine (7.5mM) and GINI treatments were performed in triplicate resulting in a total of 144 samples that were hybridised to Illumina HT12 v3 expression arrays. BeadStudio, the R-Bioconductor package Limma and GeneSpring 10.0 were used to transform the data and determine significant differences after GINI.

To identify candidate genes, each sample was compared to itself before and after NMD inhibition with caffeine treatment. Candidate ‘BRCAx’ genes were identified by comparing affected and unaffected individuals within families, and by subtracting “global caffeine response” genes that were upregulated following GINI in all samples from the candidate gene list. Current analyses have identified a total of twenty-five candidate genes: six in family A, six in family B, and thirteen in family C. Sequencing analyses will soon be in progress to determine whether these genes carry nonsense mutations in the relevant families, and if so, whether they segregate with disease. If we find putative BRCAx genes in these families, then we will screen them in ~380 case BRCAx index case DNAs and a similar number of controls.
HIGH-THROUGHPUT AND SENSITIVE MUTATION ANALYSIS OF 19 HUMAN ONCOGENES IN FAMILIAL BREAST TUMOURS USING THE ONCOCARTA™ ASSAY PANEL V1.0

Cameron N. Johnstone1,2, Nic Waddell1, kConFab2, Darryl Irwin3, Georgia Chenevix-Trench1

1) Queensland Institute of Medical Research, Brisbane, Queensland
2) Peter MacCallum Cancer Centre, Melbourne, Victoria
3) Sequenom, Inc.

Breast cancer is a heterogeneous disease with many clinically relevant subtypes. Familial breast tumours occurring in women with a germline BRCA1 mutation are preferentially basal-like, while BRCA2 breast tumours are predominantly luminal. With the aim of better characterizing molecular genetic lesions in all three familial subtypes, we employed high-throughput mutation analysis using the OncoCarta™ Assay Panel v1.0 (SEQUENOM, Inc.). The OncoCarta™ panel enables simultaneous assessment of over 230 specific point mutations in 19 human oncogenes using only 480 ng of input genomic DNA per sample. For each sample, PCR and primer extension reactions are conducted across a total of 24 multiplexes. Reaction products are then resolved using MALDI-TOF mass spectrometry (MassARRAY Compact Analyzer, SEQUENOM, Inc.).

We screened 43 familial (15 BRCA1, 13 BRCA2, 15 BRCAx) breast tumours, both fresh frozen and FFPE, all with at least 75% neoplastic content, with the OncoCarta v1.0 panel. Spectra were analyzed using Typer 4.0® software, with the mutation proportion threshold set at 10%. Expression profiling had shown that there are 12 luminal A, 6 luminal B, 19 basal-like, 5 HER2, and 1 normal-like tumours in this cohort. As expected, we identified frequent mutations in PIK3CA, particularly in luminal tumors, with the proportion of mutant alleles in each sample at 26-43% (mean 31%). In addition, 3/43 tumors had D842V mutations in PDGFRA in 13-18% of cells, and one of these three tumors also carried a T992I mutation in MET (in 34% cells), and an insertion in EGFR (in 19%). Interestingly the three tumors with atypical mutations were all basal-like tumors (2 BRCA1 and 1 BRCA2). We have also carried out copy number and expression profiling of this cohort of tumors and find that, while gene amplification of these loci is very rare (only 1/43 with EGFR amplification), the expression of MET and EGFR is significantly higher in basal-like vs luminal A and B tumors.

These data suggest that basal tumours may carry a broader spectrum of somatic mutations than luminal tumours, some of which may render them suitable for targeted therapies. In addition, our expression profiling data indicate that some of these oncogenes might be activated in basal-like tumors by other mechanisms, in addition to somatic mutation.
kConFab: 12 YEARS ON….

Heather Thorne, Eveline Niedermayr, Amber Willems, Lynda Williams, Daniela Surace, Lana Djandjgava, Kirsty Baron and the kConFab research nurse’s on behalf of the Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab).

kConFab, Research Department Peter MacCallum Cancer Centre, St. Andrew’s Place, East Melbourne, VIC, 3002.

kConFab, the Australian/New Zealand consortium for research into families at high risk of breast and ovarian cancer, has completed collection & recruitment of 1,319 families since its inception in 1997. Biological material and genetic, epidemiological, and psychosocial data are collected from affected and unaffected, female and male participants over the age of 18. This material is available to researchers that have obtained peer reviewed, ethically approved and funded research projects. At present, kConFab supplies material to more than 34 research projects world-wide.

The kConFab biological repository contains blood specimens from a total of 11,851 participants. The standardized kConFab blood processing protocol produces fractions of plasma, non lymph, blood pellet and white blood cells. White blood cells undergo EBV transformation to produce cell lines which are used by researchers in functional assays or as a replacement source of DNA/RNA. To date 1,174 EBV cell line transformations have been performed.

As of June 2009, 1,246 families have had genetic testing identifying 38% of families with a pathogenic, large genomic rearrangement (LGR) or splice site mutation in either BRCA1 or BRCA2 (54% and 46%, respectively). An additional 11% of families carry an unclassified variant in BRCA1 or BRCA2; with a further 0.8% with pathogenic mutations in the ATM, CHEK2 or TP53 genes. Family genotyping has identified 2,152 mutation carriers who carry a pathogenic, LGR or splice site mutation in BRCA1 or BRCA2 (73% female and 27% male). Of the 1,564 female participants who harbour the germline mutation, 66% are affected with breast or ovarian cancer.

The kConFab Tissue Bank currently averages four tissue collections per week. kConFab has collected 756 fresh tissue collections, consisting primarily of breast and ovarian tissue, with a small proportion of other normal and tumour tissues, such as prostate, colorectal and bone. In most cases tumour tissue collections are matched with normal tissue from a distal area of the specimen. In addition to tumour collections, a large proportion of prophylactic mastectomy and oophorectomy specimens are also collected. Consequently, tumour tissue comprises 26% of the bank, while normal tissue comprises 74%. Following collection, a full research pathology review is conducted using a standardised scoresheet for normal and tumour tissues. Important features such as percentage tumour, normal epithelial, lymph and necrotic components are scored. Furthermore, kConFab has constructed 8 tissue microarrays (TMAs), containing between 52-127 cores per array. At present ~1,000 archival blocks are being requested from pathology centres nationwide for 2 major TMA projects: 1) the CIMBA project, which focuses on known kConFab BRCA1 and BRCA2 mutation carriers; and 2) the BCAC project, which focusing on BRCAX cases. Each tumour array will consist of sixty 1mm diameter cores, with 3 replica blocks per array. Where possible, tumour cores will be matched to normal from the same archival block.

The kConFab resource enables researchers to answer important questions relating to familial aspects of breast cancer. Information about the kConFab resource and the application process is available on the web site (http://www.kconfab.org).
THE BREAST CANCER FAMILY REGISTRY

Melissa Southey and David Goldgar for the BCFR

The Breast Cancer Family Registry (BCFR), is a six-site international consortium funded in 1995 by the National Cancer Institute under a cooperative agreement (RFA CA-95-003). The BCFR by design was created as a resource for the entire scientific community to a) facilitate interdisciplinary research on the causes of breast cancer through multi-site data collection of breast cancer families recruited at both high-risk clinics and population-based settings; b) encourage translational research; and c) identify populations at high risk for breast cancer that could benefit from new preventive and therapeutic strategies. Data collected include detailed pedigree information, epidemiological data, treatment data, pathology specimens from affected participants, and blood and/or buccal specimens from all participants. The six participating centers from the USA, Canada, and Australia ascertain families either through incident breast cancer cases identified from population-based cancer registries or through clinical and community settings.

The registry now consists of more than 13,000 probands and 20,000 relatives, 20,000 blood samples, 8,000 lymphoblastoid cell lines, and tumor slides or tumor blocks for approximately 5,000 breast cancer cases and are available to the research community via a prescribed application process. Since 2005 support has continued for the core infrastructure at each site and to maximize its use by interdisciplinary teams of researchers.

The six registries of the BCFR are highly integrated in a complementary and synergistic manner. Common elements used at all sites include: baseline epidemiology, family history, and treatment questionnaires; biospecimen collection, including blood or buccal samples and tumor tissue; biospecimen processing, including cell transformation, pathology review and slide preparation, and aliquoting and storage protocols; coordinated, systematic, and quality-controlled procedures for mutation testing; and follow-up questionnaire and protocol for updating personal and family history data. The registries, individually and combined, provide a great range of resources, expertise, and specialized skills.

1. The six registries that make up the BCFR are;
- Cancer Care Ontario - Ontario site of the BCFR (PI: Irene Andrulis, Ph.D.),
- Northern California Cancer Center - Northern California site of the BCFR (PI: Esther M. John, Ph.D.),
- The University of Melbourne - Australian site of the BCFR (PI: John Hopper, Ph.D.),
- Fox Chase Cancer Center - Philadelphia site of the BCFR (PI: Mary Daly, M.D., Ph.D.),
- Columbia University - New York site of the BCFR (PI: Mary Beth Terry, Ph.D.) and the Huntsman Cancer Institute - Utah site of the BCFR (PI: Saundra Buys, M.D.).
The Rainbows for Kate Australian Sarcoma Kindred Study

Mandy Ballinger¹ and David Thomas¹²
Australasian Sarcoma Study Group¹
Sarcoma Genomics & Genetics Laboratory²,
Peter MacCallum Cancer Institute,
Melbourne, Australia

Sarcomas are a diverse set of lethal cancers that particularly affect the young. There are over 130,000 new cases worldwide each year, accounting for approximately 1-3% of all malignancies, with incidence increasing. Sarcoma has a strong genetic component and is more common in persons with recognized hereditary cancer syndromes although little data on familial risk are available in adult-onset sarcoma. Current advice regarding genetic risk in sarcoma is largely based on data from family cancer registry studies which are over represented in common cancers with well defined genetic risk and modifiable outcomes. It is by no means clear that the same genotypes will operate in families with different cancer types. In the past, familial sarcoma studies have tended to be heavily biased towards paediatric populations. Paediatric sarcomas are biologically and pathologically different to adult-onset sarcomas. This project aims to create a vital research resource to enable further study into the genetic factors contributing to the hereditary risk of developing sarcoma as an adult. Participants will be identified at one of five key sarcoma clinics in Victoria, Queensland and New South Wales, asked to complete a questionnaire and provide biospecimens (blood and tissue). The biospecimens will be stored indefinitely in a tissue bank to be utilised by researchers investigating genetic factors contributing to cancer. Information obtained from the questionnaire will be used to ascertain the family history of cancer and other factors that may contribute to development of the disease. The ongoing nature of this project will enable continual provision of important practical information for clinicians and patients leading to more favourable outcomes.
#37
MAINTAINING SAMPLE QUALITY AND AVAILABILITY IN LARGE POPULATION-BASED EPIDEMIOLOGY RESEARCH PROGRAMS

Melanie Lazareviez, Christopher Schroen and Melissa Southey for the Genetic Epidemiology Laboratory’s Biorepository*.

Genetic Epidemiology Laboratory and *The Biorepository, Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Victoria, Australia.

Large population-based epidemiological studies require well maintained, high quality bioresources to effectively measure a broad range of genetic and molecular factors. There are many components of biospecimen collection that need to be planned and synchronised to ensure high quality biospecimens are collected, transported, processed and stored. To be affective these activities need to be followed by adequate retrieval and dispatch processes - and all require accurate data management.

What biospecimens are collected and stored is usually driven by the current/initial research study focus, the level and scope of available funding/support and discipline interests of the researchers. We have found that via consultation and discussion with researchers the Biorepository is able to prepare biospecimen that can accommodate a wide range of investigations for immediate and future use.

Monitoring of sample quality extends across the entire biorepository’s processes and begins with the planning of specimen collection, specimen collection, preliminary processing (if required), transport, processing procedures, storage environment and mobilising procedures and processes.

The Biorepository, The Centre for Molecular, Environmental, Genetic and Analytic Epidemiology (The University of Melbourne) and The Cancer Council of Victoria have a long history of undertaking specimen collection and processing as an important component of large epidemiological studies that have required a high degree of organisation and coordination between centres, research participants and collection agencies.
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