Clinical management of women in BRCAX families: issues and controversies
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A1

The role of the familial cancer clinic (FCC) is to provide a cancer risk assessment and appropriate cancer risk management advice, but there are certain groups of patients for whom there are no standard risk management guidelines. One such group is women with a strong family history of breast cancer but BRCA genetic testing has not found a germline mutation. As a family history of breast cancer is the commonest reason for referral to FCCs, this clinical scenario is a frequent challenge to us all. This presentation will provide a summary of the literature surrounding:
• Breast cancer risk in BRCAX families
• Ovarian cancer risk in BRCAX families
• Does the presence of male breast cancer affect the breast and ovarian cancer risks for women in BRCAX families

Polyposis syndromes– what to do when genotyping seems not informative
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A2

Background: In polyposis, the type and numbers of polyps, the pattern of inheritance, and any extraintestinal features are all important in strategic planning of the DNA mutational approach. But where should we go when no mutation is uncovered? This paper outlines an approach we have adopted at RMH.

Multiple adenomatous polyposis: As a routine, we check to see if both sequencing and MLPA have been complete for APC, and full sequencing for MYH (though the return outside the three common mutations is low in our ethnic mix). On at least one occasion, a change in the MLPA kit by Holland Inc lead to identification of an important deletion as the probes changed in the MLPA kit. So recollecting blood to run on a new kit can be informative. RNA studies may also identify expression perturbations leading to closer scrutiny of the DNA. We then look for translocations by FISH analysis. If all this is negative, we move the patient to a research setting; Justine Marum is well into a PhD evaluating the role of AXIN mutations in this group of patients. The findings to date are being confirmed with other approaches to establishing pathogenicity of the AXIN variants identified. This work is being done also in collaboration with Dr Marie Faux at the Ludwig Institute of Cancer Research and Prof Rodney Scott at University of Newcastle.

Clinically, patients with multiple adenomas even without a germline mutation identified, are at increased risk of colorectal cancer; indeed, multiplicity of adenomas is the highest risk factor of subsequent colorectal cancer identifiable. The new NHMRC guidelines out for public comment recommend follow up of patients: As multiplicity of adenomas is a strong determinant of risk of metachronous advanced and non advanced neoplasia, follow up should be at twelve months for those with five or more adenomas, and sooner in those with ten or more adenomas.

The risk of colorectal cancer to relatives of patients with multiple adenomas is related to the number of adenomas in the proband – outside FAP and MYH. Therefore, as distinct from patients with small numbers of adenomas, relatives of patients with larger numbers (10+) should be offered colonoscopy at an age relative to the age of presentation of the proband. The interval is not well defined but I would suggest 5 yearly.

Hyperplastic polyposis: HPS has no defined genetic basis as yet. These patients are at high risk of colorectal cancer, either through the serrated pathway or through their concurrent adenomas. Two yearly colonoscopy is advised. Although the mode of inheritance is also poorly defined, it is thought it may be recessive. Therefore siblings are offered surveillance, though usually not expected to be found with HPS themselves. There is however, a high risk of colorectal cancer in first degree relatives (siblings or parents) so colonoscopy should be offered to these relatives 5 yearly.

Low level mosaicism, gonadal mosaicism, somatic mosaicism: Somatic mosaicism is now well described in FAP, with respect to the APC gene; cases of multiple adenomatous polyposis may therefore be found with no germline (lymphocytic) APC mutation. We have been searching for the same APC mutation in multiple polyps, and in background mucosa, in these patients without definitive progress, but others have reported this phenomenon. Some of this can be traced to low level mosaicism including even in the germline (lymphocytes) but not detected with standard approaches to APC mutational analysis. We have also been interested to seek evidence of gonadal mosaicism in the parents of apparent de novo cases, and are still pursuing this possibility. This requires predictive DNA testing of all siblings of apparent de novo cases, and their parents. Gonadal mosaicism in FAP has been reported in Utah. Recently we have had occasion to consider whether mosaicism may involve both the gonads and the colon – through a request to colonoscopy parents who tested negative for an apparent de novo APC mutation in their only child. This has lead to a range of opinions and experiences described from colleagues around the world which will be of interest to the FCC counsellors.
A3
Inherited and de novo germline TP53 mutations in adult-onset sarcoma
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A3

The International Sarcoma Kindred Study (ISKS) was initiated in 2009 to
to investigate the prevalence and nature of heritable risk in sarcoma
populations. Sarcomas affect a younger population than most cancers, and
are associated with some of the most striking known familial cancer
syndromes. For example, the Li-Fraumeni syndrome (LFS) is a devastating
heritable condition, conferring a 90% lifetime risk of cancer and a 50% risk
of invasive cancer by age 30. LFS is predominantly due to germline
mutations in TP53 and CHEK2. Sarcomas collectively comprise the most
frequent cancer seen in LFS. The ISKS is recruiting all adult patients
diagnosed with sarcoma in Australia, regardless of family history. Recently,
ISKS opened sites in New Zealand and India, and plans to open in
France later this year.

At June 2011, the ISKS has recruited over 450 adult sarcoma probands
and their families. The average age of onset of sarcomas in these families
is significantly younger than for sarcomas in the general population (47y
versus 59y in Victorian Cancer Registry). Non-sarcoma cancers in these
families also occur at a younger age than in the VCR (58y versus 65y,
P<0.001). A survey of recognisable familial cancer patterns identified 56%
without a family history, 23% conforming to the Eeles’ criteria for LF-like
syndrome (LFL), and 1% (4 families) with classic LFS. Another 14% of
families have odd patterns of familial clustering without conforming to
any known syndrome. There is a 2.7-fold excess of cases of Hodgkin’s
lymphoma (HL), which is not explained by radiation exposure. The age of
onset of the HL cases is 31y (compared with 42y in the VCR), while the
age of onset of the sarcomas in the HL families is 40y.

A survey is being conducted of the incidence of mutations in TP53 in
unselected patients with sarcoma to determine a) the frequency of true
LFS; b) the incidence of unsuspected de novo mutations. A combination of
sequencing (exons 2-11) and multiplex ligation-dependent probe
amplification (MLPA) was used to detect mutations in TP53. In addition,
CHEK2 exon 9 deletions and the del1100C variant were screened. Full
pedigree and pathologic information was available on all subjects. In the
first 272 probands, 12 cases (4%) of pathogenic mutations in TP53 were
identified. The majority of mutations affected the DNA binding domain,
but one mutation affected exon 2 (frameshift) and one the tetramerization
domain. We have identified only one mutation by MLPA. Four families
fulfilled criteria for LFS, two met the Eeles’ criteria for LFL, and in 6 cases
there was no family history. The pathogenicity of these apparently de novo
cases is supported by the fact that 4 individuals without family history had
two or more cancers, mostly outside a radiation field. The average age of
first cancer onset in affected individuals is 40 years, or 15 years younger
than the ISKS cohort. One further individual, who lacked a family history
but developed two cancers, carried a separate mutation in CHEK2.

We conclude that perhaps one in twenty-five individuals with adult-onset
sarcoma carries a pathogenic germline mutation in TP53 or CHEK2.
Strikingly, over half of the affected do not have a family history. These
findings have immediate clinical management implications regarding
screening, treatment and reproductive choices in the sarcoma population.
We propose to conduct a prospective study of cancer screening in
families affected by inherited or de novo mutations in TP53, in order to
develop an evidence-based risk modification program for affected
individuals.

A4
I just did it for the kids: mothering in the context of living with an
increased risk of ovarian cancer
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A4

Hereditary breast and ovarian cancer syndromes are rare genetic
disorders conferring a significant lifetime risk of developing breast and/or
ovarian cancer. Women with a strong family history of breast and ovarian
cancer face decisions regarding genetic testing, cancer surveillance and
risk reducing surgery.

This paper draws on interview data from a group of thirty-two Pakeha
New Zealand women living with an increased risk of breast and ovarian
cancer.

"Getting on with it" emerged as a dominant theme, as the way in which
most of these women approached their risk. "Getting on with it" appears
to be a deeply entrenched social, cultural and gendered expectation in
New Zealand, perhaps influenced by our history as a settler society and
the more recent influences of neo-liberal governance. This approach to
risk is influential in guiding the management decisions that these women
are making.

Mothering is central to the identity of many of the participants in this
study. They mother their children within the context of the increased
cancer risk. These women identify a strongly felt responsibility to be
there to care for their children. Alongside a strongly voiced desire to "get
on with it", they use their role as mothers to motivate their decisions
regarding risk reducing surgery. I argue that choosing to undergo the
removal of healthy body parts in order to reduce risk and remain alive to
fulfil role expectations provides a symbolic and gendered representation
of women as carers and nurturers.

A5
Are women at high risk for serous gynaecological cancer (SGC) opting
for risk-reducing salpingo-oophorectomy motivated by high levels of
anxiety and risk perceptions?
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A5

Background: This study assessed sociodemographic, biological and
psychosocial determinants of the decision to undertake risk-reducing
salpingo-oophorectomy (RRO).

Methods: Women participating in the kConFab Clinical Follow-up and
Psychosocial studies who were at increased risk for serous gynaecological
cancer (SGC) (i.e. BRCA1 or BRCA2 carriers or a family history with at least
one first- or second-degree relative with SGC), had no personal history of
cancer and had not had an RRO at the time of kConFab enrolment were
included in the analyses. Women who had been informed that they did
not carry the BRCA1 or BRCA2 mutation segregation in their family (true
negatives) were excluded. Predictor variables were assessed using self-
administered questionnaires and interviews at the time of enrolment, and
data on RRO uptake was from the most recent three-yearly follow-up
assessment.

Results: 579 women were eligible. Mean age was 43.5 years (range 18 to
74 years). 118 women (20.4%) reported having been tested and knowing
that they are mutation positive, while 461 (79.6%) reported not having
been tested. 69 women (11.8%) had an RRO during the follow-up period
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that they are mutation positive, while 461 (79.6%) reported not having
been tested. 69 women (11.8%) had an RRO during the follow-up period.

Conclusions: These findings are reassuring as they show that women’s
decision-making about RRO is associated with sociodemographic
characteristics and women’s knowledge about their carrier status, rather
than high levels of anxiety and perceived risk. The limitations of this
study include the fact that the psychological variables were assessed in
some cases several years prior to RRO, and these variables might have
been different just prior to RRO.
A6
Rehabilitating the sick role: post-surgical experiences of high risk women who undergo risk reducing mastectomy

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2)A6

Much has been written about the impact of risk-reducing breast and ovarian surgery on quality of life and high-risk women’s surgical decision-making, but much less is known about how this group experiences these elective procedures. In this paper we describe the ways in which women who have undergone risk-reducing breast surgery (+/- ovarian surgery) describe their surgical experiences. Data was collected during in-depth interviews with 21 Australian women from the KConFab Psychosocial study who had undergone risk-reducing mastectomy in the previous three years. Interview questions centred on decision-making, information needs, perceived costs and benefits of surgery, risk perception, pre-surgery expectations and knowledge, the surgery experience and convalesce, and overall satisfaction with surgical decision. When describing their experiences of surgery and convalesce women drew on two main narratives in which they described the immediate impact of surgery on convalesce and either embraced or vigorously rejected the sick role (Parsons, 1951). The extent to which women appeared to accept/reject the sick role appeared to be related to the amount of support/lack of support they received from families, friends and healthcare professionals. We conclude by arguing that the concept of the sick role can provide us with some insight into high-risk women’s experience of surgery and convalesce.

A7
Use of SDHB immunohistochemistry to identify germline mutations of SDH genes

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2)A7

Pheochromocytomas and paragangliomas occur sporadically but are commonly associated with the von Hippel Lindau (VHL) syndrome, multiple endocrine neoplasia type 2 (MEN2), neurofibromatosis type 1 (NF1) and germline mutations of succinate dehydrogenase B (SDHB), C (SDHC) or D (SDHD). It is therefore recommended that genetic testing be considered if not performed in all cases of even apparently sporadic pheochromocytomas or paragangliomas. Recently it has been demonstrated that immunohistochemistry (IHC) for SDHB is negative in all SDH mutated paragangliomas regardless of whether the B,C or D subunit is involved. Furthermore some clearly syndromic paragangliomas without known genetic mutation (including but not limited to those which occur in the Carney Triad) are identified by negative staining for SDHB [3]. Although historically the renal tumours occurring in the setting of SDHB mutation were usually classified as conventional clear cell carcinoma or oncocytoma, they actually display a unique morphology (unrecognized until know) and which can be confirmed by immunohistochemistry. The GISTs occurring in SDH mutation and Carney Triad are also unique and demonstrate quite a different morphology, natural history and molecular pathogenesis compared to other GISTs occurring in adults (but similar to most GISTs occurring in childhood). We call this unique subtype of GIST the type 2 GIST. Briefly type 2 GISTS arise in the stomach, show an epithelioid morphology, are often multifocal, commonly show lymph node metastasis, are wild type for KIT and PDGF, have a prognosis not predicted by size and mitotic rate, never respond to imatinib but demonstrate an indolent growth despite the presence of frequent metatases [3,5]. We recommend that all paragangliomas, GISTs which potentially display type 2 morphological or clinical features and renal carcinomas which display the unique morphology we described should undergo immunohistochemistry for SDHB. Negative staining for SDHB indicates an abnormality of the mitochondrial complex 2 and is an absolute

Table 1(abstract A7) Syndromes associated with paraganglioma and pheochromocytoma

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Incidence</th>
<th>Clinical syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraganglioma Syndrome type 1 (PGL1)</td>
<td>SDHD(11q23)</td>
<td>???</td>
<td>Pheochromocytomas/Paragangliomas</td>
</tr>
<tr>
<td>Paraganglioma Syndrome Type 2</td>
<td>SDHAF2</td>
<td>Extremely Rare</td>
<td>Head and neck paragangliomas</td>
</tr>
<tr>
<td>Paraganglioma Syndrome Type 3 (PGL3)</td>
<td>SDHC</td>
<td>??? (Rare)</td>
<td>Head and neck paragangliomas</td>
</tr>
<tr>
<td>Paraganglioma Syndrome Type 4 (PGL4)</td>
<td>SDHB</td>
<td>???</td>
<td>Pheochromocytomas/Paragangliomas</td>
</tr>
</tbody>
</table>

Increased risk of malignant behaviour
Most common locations:
1. Intraabdominal extra-adrenal
2. Adrenal
3. Head and neck
4. Thorax
Type 2 GIST Renal Tumours
Type 2 GIST Renal Tumours
Epidymal/broad ligament papillary cystadenomas
Medullary thyroid carcinoma
Phaeochromocytomas
Parathyroid hyperplasia
Neurofibromatosis type 1 Neuroradioblastoma
Café au lait spors
Gliomas
Lisch Nodules
Phaeochromocytomas/Paragangliomas
Most common locations:
1. Head and neck
2. Adrenal
3. Intraabdominal extra-adrenal
4. Thorax
Type 2 GIST Renal Tumours
No known familial case
No known mutation
Extremely rare
1. Paraganglioma
2. Type 2 Gist
3. Pulmonary chondroma
4. Oesophageal leiomyoma?
5. Adrenal adenoma?
indication for formal genetic testing. We perform and interpret SDHB immunohistochemistry of archived formalin fixed paraffin embedded tissue in a manner analogous to MSI testing in colon cancer. In the setting of paraganglioma or renal carcinoma negative staining almost always indicates germline SDHB,SDHC or SDHD mutation (greater than 90% chance) but may indicate Carney Triad. In the setting of GIST, Carney Triad is more likely, but SDHB, SDHC or SDHD mutation accounts for at least 25% of type 2 GIST.

The mitochondrial complex 2 links the Krebs cycle and the electron transport chain and is illustrated below:

**References**


Schwannomatosis is a form of Neurofibromatosis type 2 (NF2) characterized by multiple schwannomas without vestibular involveent, affecting the cranium, spine and periphery. Several recent genetic studies have implicated the *SMARCB1/INI1* tumour suppressor gene in familial schwannomatosis. *SMARCB1* is located centromeric to NF2 on 22q and loss of function of *SMARCB1* is also a hallmark of malignant rhabdoid tumour (MRT), a highly aggressive tumour of infancy. Both familial and sporadic schwannoma tumours show a mosaic pattern of *SMARCB1* protein expression, suggestive of tumour cells either haploinsufficient, or null for *SMARCB1* protein. Familial schwannomas linked to constitutional *SMARCB1* mutation can also have somatic mutation of NF2, and conversely, schwannoma tumours associated with constitutional NF2 mutation show mosaic loss of *SMARCB1*, suggesting the involvement of a four-hit mechanism. Molecular analysis for evidence of constitutional *SMARCB1* mutation is important in both familial and sporadically occurring schwannomatosis because of the transmission risk for a mutation predisposing to the incurable MRT, in early childhood. However as for NF2, recent evidence suggests that constitutional *SMARCB1* mutations have variable penetrance and exhibit mosaicism, highlighting the importance of examining multiple tumour tissue samples as well as blood in affected individuals to ascertain germline predisposition and to provide accurate counseling for transmission risk. Further studies are needed to define the *SMARCB1* mutation spectrum in schwannomatosis and to dissect the strikingly different biology between schwannoma and MRT.
A9
An update of clinical issues from InSIGHT 2011

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A9

The fourth biennial InSIGHT meeting, held from 30th March to 2nd April 2011 in San Antonio Texas was a great success. The meeting was very well organized by hosts Dr Patrick Lynch and Dr Michael Rodriguez-Bigas. Topics covered known genetic predispositions to gastrointestinal and other cancers, identification, risk stratification and surveillance and new developments in basic science.

A10
Selecting women for breast cancer chemoprevention and what agents should be used

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A10

Recent evidence on the chemoprevention of breast cancer comes from a number of sources:

1) Recent data on two new SERMs – lasofoxifene and arzoxifene – suggests they might be an attractive option, but further details are needed to fully evaluate their role.
2) Update on the tamoxifen and raloxifene preventive trials have shown a continued benefit after treatment completion, and an overview is ongoing to combine all the post-treatment data on risks and benefits. Latest results from the STAR trial indicate that raloxifene is less efficacious than tamoxifen, but has fewer side effects. A full new overview is in progress.
3) A report from the ATAC trial indicating that the occurrence of endocrine symptoms predicts response to both tamoxifen and anastrozole may also have relevance in the preventive setting.
4) Long term follow-up of the adjuvant trials using aromatase trials has provided new evidence on the duration of a potential preventive effect by looking at isolated new contralateral tumours. This has been an excellent model for tamoxifen and may well also be so for the AIs.
5) A very recent report from the MAP3 (Goss, NEJM,2011) indicates that after 35 months median follow up, a 60% reduction in new tumours was seen, confirming predictions made from contralateral tumours in adjuvant trials.
6) New data confirm the long term protective effect of aspirin on breast cancer, but suggests it needs to be taken for at least 5 years to get a benefit. Mixed data have appeared for the statins.

Reference


A11
Association of tamoxifen use and reduced risk of contralateral breast cancer for BRCA1 and BRCA2 mutation carriers

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A11

Background: The efficacy of tamoxifen as a breast cancer (BC) prevention strategy for BRCA1 and BRCA2 mutation carriers is uncertain.

Patients and methods: Female BRCA1 and BRCA2 mutation carriers, with a personal history of BC since 1970, enrolled in any of the BC family studies, kConFab (Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer), IBCCS (International BRCA1 and BRCA2 Carrier Cohort Study), or BCFR (Breast Cancer Family Registry) were eligible. Those with bilateral disease at first BC diagnosis, tamoxifen use prior to their first BC diagnosis, or another invasive cancer with were excluded. Data were self-reported at entry into the cohort and at follow-up. Hazard ratios (HRs) for development of contralateral BC associated with tamoxifen use after first BC diagnosis were estimated using Cox proportional hazards, modeling time from diagnosis of first primary BC, adjusting for year of birth (continuous), age at diagnosis (continuous), country and bilateral oophorectomy (yes/no, time-varying). Data were censored at contralateral mastectomy, death or loss to follow-up.

Results: Of 1642 BRCA1 and 919 BRCA2 mutation carriers, 374 (23%) and 444 (48%), respectively, took tamoxifen after their first BC diagnosis. During 21,344 person-years of follow-up, 596 contralateral BCs were observed. Overall, the adjusted HR estimates were 0.31 (95% CI: 0.22-0.45) and 0.24 (95% CI: 0.16-0.35) for BRCA1 and BRCA2 mutation carriers, respectively. After left-truncating the analysis at time of recruitment, the adjusted HR estimates were 0.52 (95% CI: 0.26-1.04) and 0.39 (95% CI: 0.17-0.89) from studying 629 BRCA1 and 412 BRCA2 mutation carriers, respectively, with 4,869 person-years of follow-up.

Conclusions: Although biased estimates due to non-random use of tamoxifen cannot be excluded, these results are consistent with tamoxifen reducing BC risk for both BRCA1 and BRCA2 mutation carriers.

A12
Bowel cancer chemoprevention – ready for the clinic?

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A12

In 1988 Gabriel Kune of Melbourne published a seminal paper reporting a negative association of the use of non-steroidal anti-inflammatory drugs such as aspirin and colorectal cancer. This prompted a deluge of case control and cohort studies, all but one of which confirmed the finding. The conclusion of these observational studies was that regular aspirin users had half as many cancers. Four randomised controlled trials (RCTs) using adenoma prevention as their endp

...
gene carriers between 18 and 60 with treatment for a minimum of 5 years and unlimited follow-up. In our present state of knowledge a pragmatic position is to recommend immediate introduction of a low dose daily aspirin (75-100mg) and encouragement to log on to the new CAPP3 website to keep abreast of the emerging story. This dose will not preclude joining the RCT in 2012 and will allow us to explore methods to personalise the aspirin dose in future by assessment of the genetic basis of aspirin sensitivity and resistance.

A13
Risk-reducing surgery for breast and ovarian cancer risks - where are we now?
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A13

This talk will revisit some familiar issues about risk-reducing breast and ovarian surgery to manage cancer risks. I will focus upon the impact that surgery has on women's lives and discuss their information needs. My talk will be illustrated with data collected during interviews with 40 Australian women as part of the kConFab Psychosocial study (Butow et al). Two emergent themes – looking different and feeling different - captured the psychosocial impact of surgery upon the interviewees. Many of the women said they felt differently about their bodies following RR surgery. All were relieved at having removed the risk of cancer that had previously been embodied in their breasts and ovaries, however reducing risk by removing breasts and ovaries is not without costs. Interviewees reported experiencing a range of negative emotions and a series of unexpected bodily sensations following surgery and reflected upon positive and negative changes in their appearance. I will conclude that while women who undergo RR surgery are now informed about some of the sequelae, they are still not adequately prepared for the reality of undergoing this procedure. It will be suggested that, in addition to cosmetic outcomes, pre-surgical counselling needs to focus upon the experiential or sensational aspects of risk reducing surgery.

A14
"It's not even about her it's about the whole family": accounts of participation in a family cancer study
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A14

Accounts of participation in genetic research are dynamic and innately social. Participation is viewed not as an individualised act but as morally significant and involving relational responsibilities. Previous work has investigated individuals’ motivations for research participation in terms of such relational complexities. Here we explore the significance of relational responsibility in the context of participation in a familial cancer study where many family members are eligible for recruitment. We consider accounts of family (non)participation, (im)morality and (ir)responsibility. By looking at accounts we can explore the ways in which people use explanations to construct social reality. Accounts are more than disclosing internal attitudes, rather they provide claims of moral worth and participation in the social world. The accounts are taken from eight in social study made them reconsider immorality and (ir)responsibility. Previous work has

character work. As research participation was framed as beneficial to the family and others, with minimal burden to the participant, excusing and justifying non-participation was required in order to explain it. This was done through descriptions of illness, disability, relatives’ feelings of guilt and logistics. In one instance a sister’s non-participation was not justified or excused but explained it in terms of her ‘selfishness’. Reports of non-participation by relatives involved negative feelings towards them, ranging from frustration to anger. Stronger emotional responses were described when the relative was perceived as having particular responsibilities towards the interviewee or their offspring, as a parent or sibling. The familial tensions described in the accounts are part of a broader narrative of pre-existing difficulties in family dynamics. The genetic research context became another field in which these difficulties became evident or entrenched. Our findings inform our understanding of how genetic research may be experienced within a family. With participation framed as acting responsibly towards others there may be relational ramifications if individuals decline to do so. This has implications for how consent is viewed and how studies may choose to recruit participants.

A15
The experiences of research participants offered genetic test results as a result of taking part in a population based ovarian cancer research study?
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A15

Background: Although the issue remains controversial, it is generally accepted that researchers have some responsibility to notify participants of information discovered during research that has the potential to significantly affect person’s health or prevent significant harm. Whilst the issue of recontact has been broadly discussed by ethicists, researchers and clinicians, few studies have reported on participant’s experiences of the process.

The Australian Ovarian Cancer Study (AOCs), a population based study, recruited women with invasive ovarian cancer between 2002 and 2006. BRCA1 or BRCA2 mutation testing has been undertaken and women in whom a mutation has been identified, or their next of kin in the case where the women is deceased, have been notified in writing (notification letter) by the researchers about the finding of a mutation and the availability of obtaining these results through a family cancer clinic (FCC). The AOCs Psychosocial project has interviewed individuals who received notification letters.

Aims of AOCs Psychosocial study: 1. Explore individuals’ understanding and response to the information contained in the letter they received from the researchers.
2. Determine what informs individuals’ decisions about whether or not to contact an FCC and take up genetic testing information.

Results: A total of 21 in depth interviews have been undertaken to date. Participant’s response to the notification letter and their understanding of the letter varied. Some participants did not recall receiving the letter. Although many of the participants made contact with an FCC after reading the letter, the data suggest some participants were confused or did not understand the notification letter. Some expressed fear or ambivalence about the contents of the letter. In addition, the invitation to participate in the psychosocial study and resultant interview process acted as an intervention, with three participants stating receiving the letter to participate in the psychosocial study made them reconsider contacting an FCC. Whilst the primary purpose of the psychosocial interview was for collecting research data, for three participants the
At the Royal Melbourne Hospital, the Genetic Counsellors identified the desire to learn from each other, and engage in new forms of learning. Subsequently, a pilot project focusing on reflection, accountability, and experiential learning was developed. Live supervision with peers was identified as the model which fitted the learning objectives of the team. The aims of the project were to:

1. Develop a live peer supervision model relevant to Genetic Counselling practice applicable to both Board Eligible and Certified Genetic Counsellors working in Familial Cancer.
2. Develop guided post observation questions based on reflective practice.
3. Identify key learning points from the live peer supervision sessions.

Seven Associate Genetic Counsellors/Genetic Counsellors participated in the project. The first phase involved collaborative brainstorming sessions to define the purpose of the project, gain knowledge and skills relevant to supervision, set the supervision contract, and develop post-observation questions. After each live supervision session, the discussions of the observer and observee were audio recorded. The second phase of the project involved analysing the recorded discussions and identifying areas of learning, relevant to clinical practice. The observer and observee’s self-reported areas of learning were also identified. This presentation will describe the process and outcomes from these two phases of the project.

This pilot project provided the Genetic Counsellors with an opportunity to enhance clinical and supervision skills, harness the skills brought by each Genetic Counsellor, learn from another’s qualities, increase awareness of one’s own style in clinical practice, and engage in the reflective process with immediacy.

There were a number of learning areas unique to this model of supervision, including access to experiential and visual learning. Live-peer supervision may be a useful model to consider for both Associate and Certified Genetic Counsellors to engage in their continuing development and growth in clinical and supervision skills, ultimately improving patient care.

A18
A state-wide population-based program for detection of Lynch syndrome based upon immunohistochemical and molecular testing of colorectal tumours
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A18

Background: We have previously established in a large retrospective study that testing for microsatellite instability (MSI) in colorectal cancer (CRC) from patients aged <60 years was an effective first screen to identify individuals with Lynch syndrome (LS). From these findings, MSI and/or immunohistochemical (IHC) screening was recommended for all newly diagnosed CRC patients aged <60 years in Western Australia, regardless of family history of cancer. In the current study we evaluated the utility of routine MSI/IHC screening by diagnostic pathology laboratories for the detection of previously undiagnosed individuals and families with LS.

From January 2009 to December 2010, 270 tumours were tested for MSI and for expression of MLH1, PMS2, MSH2 and MSH6 using IHC. Cases showing MSI and/or loss of expression were also tested for the BRAF V600E hotspot mutation. Seventy cases were found to have MSI, of which 25 were excluded from further investigation as possible LS cases due to presence of the BRAF V600E mutation. The remaining 45 “red flag” cases were eligible for germline testing based on their MSI, IHC and BRAF status. From 26 cases tested to date, 11 germline mutations have been found. Nine were from individuals not previously recognized as LS and two were untested members from known LS families. Extrapolation of the mutation incidence (11/26, 42%) to all red flag cases (n=45) suggests that approximately 19 mutation carriers exist in this cohort. This value approximates the number of LS cases that could be expected to arise in the Western Australian population over a two-year period (n=24), assuming that 1% of all CRCs are due to LS.

Although further improvements in workflow can be made, our preliminary findings following the implementation of state-wide routine MSI and IHC testing in Western Australia indicate that the majority of LS cases are being identified.
pathways are operational in HPS but that the serrated CRC pathway predominates.

Endoscopic imaging: Considering the presumed increased risk of malignant progression of polyps in HPS, detection and removal of polyps seems necessary to prevent CRC development in these patients. Besides following general quality guidelines of colonoscopy, novel advanced endoscopic techniques, such as narrow-band imaging (NBI) may improve the detection of polyps in HPS. In addition to improved detection of polyps in HPS, accurate differentiation of HPS and SSAs, which appear endoscopically very similar, may aid the endoscopist in only removing SSAs and leaving HPS, which display comparatively lower levels of genetic mutations. We evaluated the value of NBI and autofluorescence imaging for the detection and differentiation of polyps in HPS.

A20 Serrated polyposis syndrome and colonoscopic surveillance: who is it safe to follow?
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A20

Aim: To assess colorectal cancer (CRC) risk during colonoscopy surveillance in a cohort of patients with the serrated polyposis syndrome (SPS).

Method: Colonoscopy and histology records from January 2000 to time of interview for 67 New Zealand patients, meeting WHO criteria for a diagnosis of SPS and enrolled in the Genetics of Serrated Neoplasia study, were reviewed. Polyp demographics, smoking status and family history of cancer were recorded.

Results: Of 67 patients 18 presented with CRC (mean age 57yrs, 12 Female). Only one of 18 reported a first degree relative (FDR) with CRC. Over a median follow-up of 8 years from time of surgery with an average interval of 16 months between colonoscopies, two patients developed metastatic CRC. The first patient was presented with a 150 mm sessile serrated polyp and a 100 mm adenomatous polyp. The second patient was presented with a 70 mm precancerous rectal polyp. His father (the index patient) was diagnosed with CRC at 25 years of age. The patient died from CRC at 34 years of age. The third patient was presented with a 13 mm polyp that was confirmed as CRC. His father (the index patient) was diagnosed with CRC at 35 years of age. The patient died from CRC at 43 years of age. The two other patients developed metastatic CRC: one identified at prophylactic completion colectomy and one in association with first diagnosis of SPS. The average interval between colonoscopies of 8 months. All had adenomas and one a sessile serrated polyp. No significant smoking effect was seen in any group.

Conclusion: If endoscopic control is feasible, SPS patients can be judiciously managed by frequent surveillance colonoscopy.

A21 A study of cancer risks in relatives of patients with serrated polyposis
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A21

Aims: To determine whether cancer risks for relative of patients with the serrated polyposis syndrome (SPS), are increased above that of the general population.

Methods: A total of 102 CRCs were observed in first- and second-relatives of 100 MMR gene mutation carriers, three were clinic-based probands.

Results: Of 67 patients 18 presented with CRC (mean age 57yrs, 12 Female). Only one of 18 reported a first degree relative (FDR) with CRC. Over a median follow-up of 8 years from time of surgery with an average interval of 16 months between colonoscopies, two patients developed metastatic CRC. The first patient was presented with a 150 mm sessile serrated polyp and a 100 mm adenomatous polyp. The second patient was presented with a 70 mm precancerous rectal polyp. His father (the index patient) was diagnosed with CRC at 25 years of age. The patient died from CRC at 34 years of age. The third patient was presented with a 13 mm polyp that was confirmed as CRC. His father (the index patient) was diagnosed with CRC at 35 years of age. The patient died from CRC at 43 years of age. The two other patients developed metastatic CRC: one identified at prophylactic completion colectomy and one in association with first diagnosis of SPS. The average interval between colonoscopies of 8 months. All had adenomas and one a sessile serrated polyp. No significant smoking effect was seen in any group.

Conclusion: If endoscopic control is feasible, SPS patients can be judiciously managed by frequent surveillance colonoscopy.
established. Using a cohort of 764 carriers who had a diagnosis of colorectal cancer from the Colon Cancer Family Registry, we estimated age-, sex-, country- and calendar year-specific SIRs of second primary extracolonic cancers to compare with general population. We observed statistical evidence for significantly increased risks of cancers of the stomach (SIR=5.65), small intestine (SIR=7.75), liver (SIR=5.95), kidney (SIR=8.47), bladder (SIR=7.22), breast (SIR=1.85), brain (SIR=4.36), bone (SIR=17.99) and haemopoietic tissue (SIR=3.11) in both sexes, the prostate (SIR=2.05) in males, and the endometrium (SIR=40.34) and ovary (SIR=4.20) in females.

A23
The relationship between the BRAF p.V600E mutation and a family history of CRC in the early-onset CRC cases from the Australasian Colon Cancer Family Study

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Cancer Family Registry, we estimated familial risk of CRC and possibly extra-colonic cancers in relatives. The aim of this study was to determine 1) if early-onset CRC with the BRAF p.V600E mutation is associated with a family history of CRC, and 2) if pathological features of BRAF positive CRCs associate with CRC development in relatives.

Methods: Population-based recruitment of probands into the Australasian Colon Cancer Family Study between 1997 and 2006 was undertaken for newly diagnosed CRC irrespective of any family history of cancer but limited to a first primary adenocarcinoma of the colon or rectum between the ages of 18-60yrs. Patients with Lynch syndrome and MuttY-associated polyposis were excluded from the analysis. The BRAF p.V600E mutation was determined using a previously described allele-specific PCR assay on DNA from formalin-fixed paraffin embedded colorectal cancer tissue.

Results: The average age of onset for the 709 probands (49.5% female) was 46.3yrs ± 7.9yrs (SD) with a range of 18yrs to 60yrs. A history of CRC in at least one first degree relative (FDR) or second degree relative (SDR) was reported in 39.5% (280/709) of the probands. The BRAF p.V600E mutation was present in 54/709 (7.6%) CRCs. Overall, probands with a BRAF p.V600E positive CRC were less likely to have a FDR with CRC than probands with a BRAF wildtype CRC (OR=0.42, 95%CI=0.16-1.09; P=0.07) regardless of MSI status. Among the probands with a BRAF p.V600E mutated CRC, the mean age at diagnosis was significantly older for probands with a BRAF-affected FDR or SDR (50.4 years, 95%CI=47.1-53.7) when compared to probands without a family history (44.0 years, 95% CI=40.7-47.3; P = 0.02). The odds (risk) of having a family history of CRC significantly increased 11% per year of age (OR 1.11; 95% CI 1.01 – 1.21; P = 0.02) in probands with a BRAF p.V600E mutation positive CRC. When considering only BRAF p.V600E positive CRC, a mucinous histology was increased 4.4 fold in probands with a FDR or SDR with CRC (n=17) when compared to probands without a family history of CRC (n=37), although this was not significantly different (OR=4.40; 95%CI = 0.59 – 32.62; P=0.15).

Conclusions: In this study of early-onset CRC, we describe evidence for a relationship between the BRAF p.V600E mutation and a family history of CRC that is related to an increasing age of CRC onset. These findings, in conjunction with observations suggesting certain pathological features are associated with a family history of CRC in BRAF p.V600E mutated CRC warrant further investigation.

A24
Targeting oncogenes in advanced melanoma

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The ability to target oncogenes in malignancies such as CML, GIST, APML and ERBB2-positive breast cancer has revolutionized the management of those diseases. Interesting over 70% of melanomas contain genomic amplification or mutations in one of the oncogenes BRAF, NRAS, KIT, CCND1 or CDK4 that may induce an oncogene addicted state. Inhibition of BRAF with Vemurafenib or GSK218346 in BRAF-mutant melanoma have shown responses in over 50% of patients with advanced disease and in the case of Vemurafenib striking improvements in survival compared to DTIC. These data suggest that there are therapeutically targetable oncogenes in melanoma. However emergence of resistance is common. A number of mechanisms of resistance have been identified including reactivation of the RAS/BRAF/ERK pathway. Current focus is the development of combination strategies including the addition of MEK-inhibitors to BRAF-inhibitors and the combining targeted agents with immunological agents such as Ipilimumab. Together these data indicate that targeting oncogenes in melanoma offers significant therapeutic opportunities in one of the most challenging of human malignancies for systemic therapy.

A25
Targeting BCL-2-expressing basal-like breast cancer with BH3-mimetics

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Impairment of apoptosis is a hallmark of cancer and can result in resistance to chemotherapy. Tumour resistance to apoptosis is frequently acquired through deregulated expression of BCL-2 family members or inactivation of the p53 tumour suppressor pathway. Over-expression of the pro-survival protein BCL-2 is common in breast cancer (where it is readily detected by immunostaining), and has shown to be an important prognostic marker. A potential role for BCL-2 as a therapeutic target in breast cancer, however, has not been explored. Recently, small molecules termed ‘BH3-mimetics’ have been developed that mimic the action of pro-apoptotic BH3-only proteins. These bind and neutralize pro-survival proteins including BCL-2.

Tissue microarrays containing 197 primary breast tumours were evaluated for the expression of BCL-2, its anti-apoptotic relatives MCL-1 and BCL-XL, and the pro-apoptotic BH3-only ligand BIM. These proteins were co-expressed at relatively high levels in a substantial proportion of heterogeneous breast tumours, including clinically aggressive basal-like cancers. To determine whether the BH3-mimetic ABT-737 that neutralizes BCL-2, BCL-XL and BCL-W, had potential efficacy in targeting BCL-2-expressing basal-like triple negative tumours, we generated a panel of primary breast tumour xenografts in immunocompromised mice and treated recipients with either ABT-737, docetaxel or a combination. Tumour response and overall survival were significantly improved by combination therapy, but only for tumour xenografts that expressed elevated levels of BCL-2. Treatment with ABT-737 alone was ineffective, suggesting that ABT-737 sensitizes the tumour cells to docetaxel. Combination therapy was accompanied by a marked increase in apoptosis and dissociation of BIM from BCL-2. Notably, BH3-mimetics also appeared effective in BCL-2-expressing xenograft lines that harbored p53 mutations. In summary, primary breast tumour xenograft models that recapitulate the phenotype of the primary tumour have been developed as useful ‘proof-of-principle’, pre-clinical models. Our findings provide the first in vivo evidence that BH3-mimetics can be used to sensitize primary BCL-2-expressing breast tumours to taxane chemotherapy. Our results suggest
that elevated BCL-2 expression constitutes a predictive response marker in breast cancer. These findings provide a rationale for the development of clinical protocols using the oral analogue ABT-263 (navitoclax) as an adjunct to taxane chemotherapy in BCL-2-expressing basal-like and luminal breast cancer.

A26 Inherited and de novo germline TP53 mutations in adult-onset sarcoma

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A26

The International Sarcoma Kindred Study (ISKS) was initiated in 2009 to investigate the prevalence and nature of heritable risk in sarcoma populations. Sarcomas affect a younger population than most cancers, and are associated with some of the most striking known familial cancer syndromes. For example, the Li-Fraumeni syndrome (LFS) is a devastating heritable condition, conferring a 90% lifetime risk of cancer and a 50% risk of invasive cancer by age 30. LFS is predominantly due to germline mutations in TP53 and CHEK2. Sarcomas collectively comprise the most frequent cancer seen in LFS. The ISKS is recruiting all adult patients diagnosed with sarcoma in Australia, regardless of family history. Recently, ISKS opened sites in New Zealand and India, and plans to open in France later this year.

At June 2011, the ISKS has recruited over 450 adult sarcoma probands and their families. The average age of onset of sarcomas in these families is significantly younger than for sarcomas in the general population (47y versus 59y in Victorian Cancer Registry). Non-sarcoma cancers in these families also occur at a younger age than in the VCR (58y versus 65y, P<0.001). A survey of recognisable familial cancer patterns identified 56% without a family history, 23% conforming to the Eeles’ criteria for LFS, and 1% (4 families) with classic LFS. Another 14% of families have odd patterns of familial clustering without conforming to any known syndrome. There is a 2.7-fold excess of cases of Hodgkin’s lymphoma (HL), which is not explained by radiation exposure. The age of onset of the HL cases is 31y (compared with 42y in the VCR), while the age of onset of the sarcomas in the HL families is 40y.

A survey is being conducted of the incidence of mutations in TP53 in unselected patients with sarcoma to determine a) the frequency of true LFS, b) the incidence of unsuspected de novo mutations.

A combination of sequencing (exons 2-11) and multiplex ligation-dependent probe amplification (MLPA) was used to detect mutations in TP53. In addition, CHEK2 exon 9 deletions and the dell1100C variant were screened. Full pedigree and pathologic information was available on all subjects.

A27 A multi-center study to evaluate the impact of germline BRCA1 and BRCA2 mutations on ovarian cancer survival

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A27

Background: Approximately 10 percent of women with invasive epithelial ovarian cancer (EOC) carry deleterious germline mutations in BRCA1 or BRCA2. However, the impact of these mutations on ovarian cancer prognosis remains unclear.

Methods: We performed an international multi-center study of 1,470 EOC cases with pathogenic germline mutations in BRCA1 (1,134) or BRCA2 (336) and 2,814 non-carriers. Our goal was to further characterize the survival of BRCA carriers with EOC compared to non-carriers and to determine whether BRCA1 and BRCA2 carriers show similar survival patterns. Cox proportional hazards regression, both unadjusted and adjusted for other prognostic variables, was used to measure differences in overall survival during the five years following diagnosis.

Results: The five-year overall survival was 36 percent for non-carriers, 44 percent for BRCA1 carriers and 52 percent for BRCA2 carriers. After adjusting for study and year of diagnosis, BRCA1 and BRCA2 carriers showed a more favorable survival than non-carriers (BRCA1, HR=0.78; 95% CI=0.68-0.89, P=2x10^{-5}; BRCA2, HR = 0.61; 95% CI=0.50-0.76, P=6x10^{-6}). These survival differences remained after adjustment for stage, grade, histology and age at diagnosis (BRCA1, HR=0.73, 95% CI=0.64-0.84, P=2x10^{-5}; BRCA2, HR = 0.49, 95% CI=0.39-0.61, P=3x10^{-5}).

Conclusions: We observed a significantly improved survival in germline BRCA1 and BRCA2 mutation carriers with EOC compared to non-carriers. BRCA2 carriers had the most favorable outcome with a distinct clinical course from BRCA1 carriers. The magnitude of the differences we observed highlight the need for clinical trials in EOC to be stratified by BRCA1/2 status and suggest that the routine testing of women presenting with high-grade serous EOC may be warranted.

A28 Common genomic variants associated with breast cancer predict the risk of secondary breast cancer diagnosis

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A28

Background: A diagnosis of a second primary breast cancer (‘bilateral breast cancer’) occurs in between 2-11% of women affected by breast cancer in the general population, but is increased in frequency in women with a family history of breast cancer. We investigated whether the common genomic variants described in breast cancer GWAS studies were determinants of the risk of bilateral breast cancer and compared the effects in that of family history.

Methods: The Victorian FCC Translational Breast Cancer Cohort consists of the index cases of 1374 families with a high risk family history of breast or ovarian cancer. The cohort has been extensively characterised, including detailed personal and family history and has undergone genetic testing for both high penetrance genes and low penetration variants associated with breast cancer. 1143 women in the cohort have had at least one breast cancer and 208 women have had multiple primary breast cancers. For this study genotypic data from 22 common genomic variants (SNPs) previously identified in breast cancer GWAS were analysed for each index case and compared to the same set of genotypes from an Australian population based control group (n=895, recruited from the electoral roll for the AOC5). A Polygenic Risk Score (PRS) was calculated using a multiplicative model as the sum of the log odds ratios associated with each allele. Unilateral and bilateral cases were compared on the basis of both the measured polygenic risk and family history parameters.

Results: 768 women affected by breast cancer were identified in whom BRCA1 or 2 mutation was excluded by sequencing and MLPA. The cohort was followed up an average of 8.8 years since their diagnosis of breast cancer with 135 (18%) women having a diagnosis of a secondary primary breast cancer. The average Polygenic Risk Score in the group was 1.30 (Log OR) but was significantly increased in the women with a history of bilateral versus unilateral breast cancer (1.40 vs 1.28 p=0.018), with no difference between the groups in follow up (or time to cancer diagnosis) (7.3 vs 7.8 years, p=0.50) or age of first diagnosis (44.8 vs 45.5, p=0.47).
Measures of the family history including the number of breast cancers in first degree or any relatives, family history of bilateral breast cancer, male breast cancer or ovarian cancer were also examined but showed no significant differences between women with unilateral or bilateral breast cancer.

To examine the clinical significance of the difference in PRS the cohort was divided into high, intermediate and low polygenic risk groups. The OR for bilateral breast cancer between the high and low risk groups was 1.9 (95% CI 1.07-3.49, p=0.027) and survival analysis showed a significantly increased rate of second primary breast cancer in the high versus low polygenic risk group (cumulative hazard to 20 years of 0.39 vs 0.24).

Conclusion: Analysis of polygenic risk, determined by common genomic variants, has the potential to stratify women affected by familial breast cancer in relation to their risk of second primary breast cancer, independent of measures of family history.

A29
A breast cancer prediction model incorporating familial and personal risk factors
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A29

Risk factors for breast cancer can be allocated to one of four groups: 1 Family History/genetic 2 Reproductive/hormonal 3 Proliferative benign breast disease 4 Mammographic density These four factors have now been thoroughly studied and accurate quantitative estimates for the risk are now available for many of them. The most useful summary comes from the Oxford collaboration, which has now produced a series of papers estimating risk for individual factors. Less is known about the possible interaction between these factors and virtually nothing is known about how different factors influence the risk of different types of breast cancer e.g. oestrogen receptor positive versus negative tumours. Risk factors appear to be largely independent and this facilitates building a model to predict risk for individuals. Previous models have focused on either non-genetic factors [5] where important factors relating to genetic risk are not considered, or strictly familial factors in which the modifying effect of other factors is not included [4]. Mammographic density has not been included in any of these models, although it is currently the one risk factor with the largest population attributable risk [2]. Other authors have looked at combined models however [6]. Here we briefly review the main risk factors for breast cancer and describe our own model [7] and a computer programme for synthesizing the factors into an individual risk profile. The model has novel features in terms of combining family history data using segregation analysis with phenotypic factors using the proportional hazards model. Some comparisons between models will be made [1].

References

A30
A genetic journey through cancer: from rarity to family to aspirin and nanowires
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A30

Rare diseases illuminate the most important molecular steps along cell pathways. A clinical encounter with a woman crippled by the autosomal dominant turban tumour syndrome or cylindromatosis led us to chromosome 16 and the CYLD gene, later shown to be a key regulator of ubiquitination in the NFKappa B pathway and a potential target for high dose salicylate therapy. A genome wide approach added dysregulated trypomysin kinase signalling (Trk)We developed an in vitro cylindroma primary cell culture model which permitted experimental validation of Trk inhibitors as a viable therapeutic approach. Familial studies in our centre have focused on hereditary cancer. Two large pedigrees led to collaboration with Kolodner in identification of the mismatch repair pathway and rapid entry into diagnostics which, in turn, led to development in 1994 of our international CAP22 randomised controlled trial of aspirin and resistant stanch in 1009 carriers of an MMR gene defect. We have demonstrated, in prolonged double blind follow up that taking 2 aspirins daily for 2 years results in a subsequent 60% reduction in new cancers at a mean of 5 years (paper in press). COX2 inhibition is an unlikely sole mechanism for such delayed effects. Enhanced apoptosis, similar to that seen in infected plants triggered by natural salicylates, would explain the effect. Another under-explored mechanism open to modulation is the immune response to mismatch repair deficient cells. Lymphocytic infiltration is the hallmark of these tumours and recent studies by our research partners have revealed striking levels of immune response to the frame-shift derived neoantigens released by MMR deficient cells. These antibody responses provide a credible biomarker for our planned CAP3 dose inferiority study which will examine different doses of aspirin in 3000 gene carriers. This immunological discovery also opens the way to possibility of a vaccine against the neoantigens which would have relevance for the 1 in 6 sporadic cancers which manifest acquired MMR defects. Simpler methods to investigate tumour genomes in the pathology lab are needed because while aspirin can be offered to all, most new therapies call for greater targeting. Our commercial venture, QuantuMDx hopes to fill this gap. Tethering single strand DNA to a silicon nanowire and sequencing with heavily charged chain terminating nucleotides in a microfluidic vehicle offers robust cheap genotyping “while you wait”, converting chemistry to computer code without the “middlemen”. Affordable and reliable testing for microsatellite instability and activating ras mutations could then be easily incorporated into standard histopathology practice. Proof of principle is anticipated within months and a commercial device is planned within three years. If the method proves to be as robust as we anticipate, it offers cheap genotyping in pharmacies for common variants such as the UGT1A6 polymorphisms which modulate response to aspirin and would allow more personalised dosing as we move towards a recommendation of general aspirin use in the over 50’s for prevention of vascular events and cancer.

A31
Estimation of probabilities in favour of pathogenicity for missense substitutions for use in clinical evaluation of mismatch repair gene variants
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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. Such unclassified variants (UVs) include rare nucleotide changes predicted to cause missense substitutions, small in-frame deletions, or possible alterations in splicing.

We are developing a MMR multifactorial likelihood model to provide a quantitative measure of MMR variant pathogenicity. Bayes analysis of families to measure variant causality by segregation methods is established. Likelihood ratios for microsatellite instability and somatic BRAF tumour status have also recently been incorporated into the multifactorial model. We are currently estimating the prior probability of pathogenicity for MMR missense substitutions based on the evolutionary conservation and physicochemical properties of amino acid alterations. To this end, we have built and curated multiple-species sequence alignments of the four MMR proteins. In parallel, we identified 143 apparent missense substitutions, exonic, splice site, and intronic, in the DNA mismatch repair (MMR) genes. MLH1 and MSH2 are associated with substantial clinically increased risks of colorectal cancer (CRC). MLH1 mutation carriers were found to be at increased risk of disease. A combined analysis of three of these sets was performed to better define this association.

Methods: The three populations combined totalled 1359 individuals from 425 families with a molecular diagnosis of Lynch syndrome. To date, this represents the largest Lynch syndrome cohort examined for modifier genes. Seven SNPs, from 6 different CRC susceptibility loci, were genotyped by both research groups and the data analysed collectively.

Results: Individuals with MLH1 mutations harbouring the CC (variant) genotype of SNP rs3802842 are at increased risk of CRC (HR = 2.77, p < 0.001) and develop CRC on average 11 years earlier than individuals with the AA (wild type) genotype. All females (MLH1, MSH2 and MSH6 mutation carriers) carrying the CC genotype of SNP rs3802842 are at increased risk of CRC (HR = 2.16, p = 0.005), while female MLH1 mutation carriers are at highest risk (HR = 3.88, p < 0.001).

To investigate whether a cluster of risk alleles increases the risk of CRC, SNP rs3802842 was combined with the other six SNPs additively. MLH1 mutation carriers harbouring 3 risk alleles for SNP combination rs3802842 (rs10795686 + rs16892765) display an increased risk of CRC (HR = 5.67, p = 0.001) and an immense difference in the age of diagnosis of CRC of 28 years is observed compared to individuals with 0 risk alleles. While SNP combination rs3802842 + rs10795686 (10p14) displays an increased risk of CRC for all females harbouring 4 risk alleles (HR = 5.52, p = 0.0033).

Conclusion: These results confirm the role of modifier genes in HNPCC. We recommend that Lynch syndrome patients with MLH1 mutation and all Lynch syndrome females are genotyped for two SNPs in each group so that a personalised risk assessment and tailored surveillance program can be offered to patients at increased risk of CRC and therefore likely to develop their CRCs at much younger ages than the average age of disease onset.

A33
Substantial unexplained variation in cancer risks for MLH1 and MSH2 mutation carriers
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A33

Background: Germline mutations in the DNA mismatch repair genes MLH1 and MSH2 are associated with substantially increased risks of colorectal cancer (CRC), endometrial cancer (EC) and certain other cancers. Due to the rarity of these mutations, previous studies have been underpowered to provide precise estimates of risks.

Methods: We studied 167 MLH1 and 225 MSH2 mutation-carrying families comprising 17,352 members from the Colon Cancer Family Registry. Probands were recruited either because they had a family history of cancer (n=274) or from cancer registries independently of family history (n=118). Hazard ratios (HRs) of cancer risks for carriers compared with the general population and age-specific cumulative risks for carriers (penetrance) were estimated using modified segregation analysis conditioned on ascertainment. Heterogeneity in risks for carriers was modelled with a polyclinical risk modifier (as in the BOADICEA model).

Results: The age-specific incidence of CRC for male MLH1 mutation carriers was estimated to be 222 times (95% CI: 152-324) that for the population at ages 40 years and younger but only 6.1 times higher (95% CI: 2.4-15.5) after age 60 years. This decline (p<0.0001) in the CRC HR

A32
Chromosome 8q23.3, 10p14 and 11q23.1 variants modify colorectal cancer risk in Lynch syndrome – a combined analysis of the Australian, Dutch and Polish Lynch syndrome cohorts
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A32

Background: For a decade researchers have been searching for modifier genes in individuals with a molecular diagnosis of Lynch syndrome but the task has proven difficult as discordant results seem to be the rule rather than the exception. Recently, two colorectal cancer (CRC) susceptibility loci have been found to be significantly associated with an increased risk of CRC in Dutch Lynch syndrome patients irrespective of which gene was mutated. In a combined study of CRC risk in Australian and Polish Lynch syndrome patients only MLH1 mutation carriers were found to be at increased risk of disease. A combined analysis of the three sets was performed to better define this association.

Methods: The three populations combined totalled 1359 individuals from 425 families with a molecular diagnosis of Lynch syndrome. To date, this represents the largest Lynch syndrome cohort examined for modifier genes. Seven SNPs, from 6 different CRC susceptibility loci, were genotyped by both research groups and the data analysed collectively.

Results: Individuals with MLH1 mutations harbouring the CC (variant) genotype of SNP rs3802842 are at increased risk of CRC (HR = 2.77, p < 0.001) and develop CRC on average 11 years earlier than individuals with the AA (wild type) genotype. All females (MLH1, MSH2 and MSH6 mutation carriers) carrying the CC genotype of SNP rs3802842 are at increased risk of CRC (HR = 2.16, p = 0.005), while female MLH1 mutation carriers are at highest risk (HR = 3.88, p < 0.001).

To investigate whether a cluster of risk alleles increases the risk of CRC, SNP rs3802842 was combined with the other six SNPs additively. MLH1 mutation carriers harbouring 3 risk alleles for SNP combination rs3802842 (10p14) display an increased risk of CRC (HR = 5.67, p = 0.001) and an immense difference in the age of diagnosis of CRC of 28 years is observed compared to individuals with 0 risk alleles. While SNP combination rs3802842 + rs10795686 (10p14) displays an increased risk of CRC for all females harbouring 4 risk alleles (HR = 5.52, p = 0.0033).

Conclusion: These results confirm the role of modifier genes in HNPCC. We recommend that Lynch syndrome patients with MLH1 mutation and all Lynch syndrome females are genotyped for two SNPs in each group so that a personalised risk assessment and tailored surveillance program can be offered to patients at increased risk of CRC and therefore likely to develop their CRCs at much younger ages than the average age of disease onset.
OPCML, a novel systems regulator of tyrosine kinase signaling in ovarian and other cancers

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A34

OPCML, a GPI anchored tumor suppressor gene is inactivated by somatic methylation in multiple cancers. We previously identified this gene by LOH mapping and demonstrated that it was inactivated by somatic methylation in 80% of ovarian cancers. Restoring OPCML expression by stable transfection suppressed in-vitro growth and in-vivo tumorigenesis. We hypothesized that OPCML might abrogate growth signaling pathways. In SKOV-3 and PE01, ovarian cancer cell lines with no expression of OPCML, we demonstrated that OPCML negatively regulates a specific repertoire of receptor tyrosine kinases (RTKs) EPHA2, FGFR1, FGFR3, HER2 and HER4, and reciprocally, OPCML siRNA and shRNA knockdown in normal ovarian surface epithelial cells up-regulates these same RTKs, with no effect on RTKs EPHA10, FGFR2, FGFR4, EGFR, HER3, VEGFR1 and VEGFR3. shRNA knockdown shows that loss of OPCML accelerates the growth of normal ovarian surface epithelial cells in vitro. Example immunoprecipitation experiments revealed that OPCML binds to EPHA2, FGFR1 and HER2 extracellular domains with no such interaction to EGFR, thus OPCML binds directly to RTKs that it negatively regulates. Cotransfection of ECO less and ECD containing Her2/Neu shows that the functional tumor suppressor phenotype of OPCML is mediated via interaction with the ECD. We demonstrate that OPCML is located exclusively in the raft membrane fraction and sequesters RTKs that it binds to the raft fraction, leading to polyubiquitination and proteosomal degradation via a cas-1 endosomal mechanism resulting in systems depletion of this specific RTK repertoire, that does not occur with RTKs that OPCML does not bind. Pulse-chase experiments confirm rapid loss of HER2 in OPCML expressing cells and not in OPCML deficient cells. We demonstrate that OPCML abrogates EGF mediated phosphorylation of FGR1, HER2 and EGFR and the downstream phosphosignaling of pErk and pAKT. A recombinant modified OPCML-like protein without a GPI anchor, signal peptide or glycosylation was constructed and expressed in E. coli. This rOPCML tumor suppressor protein therapeutic caused growth inhibition by apoptosis in 6/7 ovarian cancer cell lines tested with no effect on OPCML expressing normal ovarian surface epithelium by an identical mechanism to the transfected normal protein. pErk and pAKT inhibition was seen with functional growth inhibition and increased apoptosis in rOPCML treated cells. rOPCML was then injected intraperitoneally twice weekly in two murine intraperitoneal models of ovarian cancer (nude mouse A2780 and SKOV3) and demonstrated profound inhibition of tumour weight, ascites volume and peritoneal dissemination compared with BSA control. Western analysis from rescued tumors confirmed that the same mechanism of specific RTK downregulation was also evident in vivo. In Summary, the OPCML tumour suppressor mediates its suppressor function by systems level negative regulation of at least 5 RTKs and a recombinant modified derivative is a potent tumor suppressor protein therapeutic in-vitro and in-vivo that recapitulates the in-vitro mechanism.

A35
Reclassifying ovarian cancer: origins, subtypes and resistance to therapy

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A35

Recent pathological and molecular studies have forced a very significant re-evaluation of the conventional classification of EOC. Microarray and other molecular experiments demonstrate that EOC is a series of molecularly distinct diseases that individually bear more resemblance to certain non-ovarian cancers than they do to each other. Ovarian cancer really represents a spectrum of distinct diseases that share an anatomical location. The presentation focuses on the increasing understanding of the molecular differences between and within different ovarian cancer histotypes. Particular attention is given to high-grade ovarian serous cancers, which account for about two thirds of ovarian cancer deaths, and ovarian clear cell cancers, a tumour type with generally poor response to platinum-based therapy. Using both gene expression (GE) and DNA copy number (CN) analyses, we have defined novel molecular subtypes of high-grade serous cancers [1]. The molecular subtypes are robustly represented in multiple datasets and are associated with distinct clinical outcomes, and therefore appear to be biologically meaningful. Our efforts to understand the drivers of molecular subtypes of high-grade serous will be discussed [2,3]. A clear cell cohort was analysed using GE and CN analyses, demonstrating deregulation of receptor tyrosine kinases and cytokine pathways [4]. In particular, deregulation of IL6/STAT3/HIF pathway and its targeting in a clinical setting will be described. Platinum remains the mainstay of treatment for high-grade serous cancers, however, about 20% of patients fail initial treatment and of those that respond, the majority relapse within 2 years and progressively develop resistance to treatment. We have identified mechanisms of primary treatment failure [5,6] and are currently analysing paired primary and relapse samples to determine mechanisms of acquired treatment failure as part of the ICGC project. Studies in treatment resistance will be discussed.

References

A36
Identification of a genetic variant associated with treatment outcome in ovarian cancer: the potential role of cholesterol metabolism as a determinant of response to chemotherapy

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with age was also evident for female MLH1 mutation carriers (p=0.002) but was less marked. Male MLH1 mutation carriers on average had higher CRC HRs than female carriers (p=0.004). The estimated CRC HRs for MSH2 mutation carriers were similar to those for MLH1, with any differences consistent with chance (p=0.3). The estimated EC HRs also decreased sharply with age (p<0.0004 and 0.001 for MLH1 and MSH2, respectively) and no difference by gender was observed (p=0.7). There was strong evidence for an unmeasured polygenic modifier of risk (p<0.0001). The estimated average cumulative risks (95% CI) of CRC to age 70 years were 44% (35-54) for male carriers and 38% (30-48) for female carriers, and corresponding EC risks were 22% (14-31). However for carriers in the lowest vs. highest quartiles of polygenic risk (respectively) these were 4.9% vs. 93% for male CRC, 3.5% vs. 88% for female CRC and 1.5% vs. 59% for EC.

Conclusions: This international study shows that, although the average cancer risks for MLH1 and MSH2 mutation carriers are similar, there is substantial unexplained variation in risks due to differences by mutation or by genetic or environmental modifiers. This finding has implications for the counselling and clinical management of mutation carriers.
Cell-based models have shown that response to chemotherapy has a heritable component. We hypothesized that in order to identify loci associated with treatment outcome we should focus on cases known to have had uniform chemotherapy for epithelial ovarian cancer. We therefore performed a two-stage genome-wide association study (GWAS) of progression free survival (PFS) following first line carboplatin/paclitaxel chemotherapy in ovarian cancer cases. In the first stage, we genotyped (Illumina Omni1) 183 Australian cases selected using an extreme phenotype design, and also included data on 134 cases from the TCGA and 68 from Mayo Clinic. In this stage, 260 cases had uniform treatment ("primary") group: at least 4 cycles of carboplatin 5-6 AUC and paclitaxel.

Considering the clear emerging role of the DNA repair and the tumor suppressor genes involved in DNA repair pathway (in particular those involved in nucleotide excision repair, base excision repair, homologous recombination repair and BRCA/Fanconi anemia pathway) and cell cycle checkpoint pathway (CHK1) and their correlation with the clinical-pathological characteristics.

Experimental design: A single core from archived clinically annotated tumor specimens from 80 women with TNBC and 70 with LABC were performed using Tissue Micro Array machine. The mRNA expression by RT-PCR of in genes involved in NER pathway (ERCC1, XPA, XPG), in the BRCA/Fanconi anemia pathway (FANC-A, FANC-C, FANC-F, FANC-D2), in BER pathway (PARP) and cell cycle checkpoint (Chk1) were analyzed by RT-PCR. Scores of single genes were combined with clinical data to assess association with outcome (Overall Survival –OS- and Event Free Survival –PFS).

Results: Among the NER genes, ERCC1 (p < 0.0001) and XPA (p = 0.03) genes were significantly less expressed in TNBC than in LABC, among the FA genes, BRCA1 (p < 0.0001), FANCDD (p < 0.0001), FANC F (p < 0.0001) and PALB2 (p = 0.0006) genes were significantly less expressed in TNBC than in LABC. Also CHK1 was less expressed in TNBC (p < 0.0001). In relation to the clinical-pathological characteristics, lower level of XPG and FANCA were associated with larger tumor size (≥ pt2) at definitive surgery in TNBC.

The high expression of FANCA was related to an increase of either the Overall survival (p = 0.0045) or the Event-Free Survival (p = 0.0141) on univariate analysis in TNBC.

Conclusions: DNA repair and cell cycle checkpoint related genes with particular regards to ERCC1/XPA in NER family, BRCA/FANC family and CHK1 may be useful as prognostic markers in TNBC and likely to be important in familial BRCA mutated cancers accordingly to their affinity. Their determination could be relevant for clinicians in selecting the proper treatment to adopt in TNBC.
a larger set of probands with more modest family histories. Designs were evaluated using a cost function that assumed the cost of sequencing the whole exome was 400 times that of sequencing a single candidate gene. Results indicate that while requiring variants to be identified in multiple pedigrees and/or in multiple individuals in the same pedigree are effective strategies for reducing false positives, there is a danger of over-filtering so that most true susceptibility genes are missed. In most cases, sequencing more than two individuals per pedigree results in reduced power without any benefit in terms of reduced overall cost. Further, our results suggest that although no single strategy is optimal, simulations can provide important guidelines for study design. Examples in familial breast cancer and melanoma will be presented to illustrate these points.

A39
Whole genome sequencing in the study of disease and application in personalised medicine
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A39

Next Generation Sequencing has enabled a range of applications to investigate every facet of genomic science including variant detection, transcriptome profiling and epigenetic studies. Many of these applications were previously either impractical or uneconomical by Sanger sequencing. In particular, whole genome and exome sequencing are now within the reach of an increasing number of researchers due to continued reduction in costs, improvements in workflow and accessibility of appropriate technologies. Furthermore, the improvements in data handling, storage and analysis tools have contributed significantly in providing biologist-friendly methods to assemble reads and call variants. Here I will review the recent application of whole genome and exome sequencing in both clinical and research environments for the study of breast cancer. Specifically, a recent whole-genome investigation of 50 tumour-normal pairs sourced from oestrogen receptor positive breast cancers will be explored. This study confirmed genes previously implicated in this type of cancer, as well as identifying 3 new candidates: MAP3K1, ATR and MYST. To improve clinical outcomes, it is hoped that whole genome sequencing in this study will identify treatment-resistance mechanisms for cancers sourced from women resistant to oestrogen-lowering treatment. Additional case studies will be used to highlight how these techniques have advanced our understanding in determining the treatment and subsequent clinical outcomes for individuals suffering from rare diseases.

A40
Identification of new breast cancer predisposition genes via whole exome sequencing
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A40

The application of massively parallel sequencing (MPS) platforms has begun to revolutionize our understanding of the immense variation in the human genome and the complexity that can underlie genetic susceptibility to disease. The utility of exome capture MPS through the identification of genes for rare Mendelian disorders based on analysis of only a few individuals has been eloquently demonstrated. Common diseases such as breast cancer present substantially increased complexity in terms of locus, allelic and phenotypic heterogeneity, as well as complex relationships between genotype and phenotype (reduced penetrance, phenocopies etc.). With careful consideration of study design [1], thoughtfull selection of families from our international resources (whole exome sequencing of two highly selected affected members of multiple-case breast cancer families), and a well-developed strategy (analytical pipeline) for distinguishing the few true breast cancer susceptibility genes from the many genes that have rare genetic variants that could plausibly alter protein function, we are advancing a large program of work aimed at identifying the majority of the “missing heritability” of breast cancer.

Our early findings demonstrate that:
1) despite very plausible biological roles, some genetic variants in some genes predicted to be damaging by SIFT and Polyphen2 do not appear to be associated with breast cancer risk [2].
2) application of our strategy can identify new breast cancer susceptibility genes. During the early conduct of this program, we identified a family with a protein truncating mutation in a gene involved in DNA repair. Follow-up has included mutation screening of:
   a) youngest affected members of 250 multiple-case breast cancer families,
   b) cases and controls participating in Australian population-based studies of breast cancer (ABCFS and MCCS)
   c) cases and controls in an international population-based case-control family resource (BCFR).
To date, these analyses have identified 6 families with frameshift or evolutionarily unlikely missense mutations in this gene. Features of these families include multiple-cases of early-onset female breast cancer with some potentially other interesting features such as early-onset male breast cancer and pancreatic cancer. These mutations have not been found in approximately 3000 unaffected population-based controls without a family history of breast cancer.

We are continuing to expand our dataset to include the exome sequences of further families and coordinating the follow-up of candidate genes using appropriate MPS platforms and as founding partners of a newly formed international consortium of breast cancer exome sequencing researchers.

References

A41
Identification of breast cancer susceptibility genes using whole exome sequencing
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A41

Recent advances in technology have opened up the possibility of using next generation sequencing to efficiently uncover predisposing mutations in individuals with inherited cancer in an unbiased manner. We are conducting whole exome sequence analysis of germline DNA from multiple affected relatives from breast cancer families with the aim of identifying rare protein truncating and non-synonymous variants that are likely to include novel cancer predisposing mutations. Data from >70 exomes show that on average each individual only carries 30-50 protein truncating mutations and 300-400 rare non-synonymous missense variants. By considering only those variants shared by multiple affected relatives the number of candidate predisposing mutations can be dramatically reduced to just 3-5 truncating mutations and 10-20 non-synonymous variants per family. Among the first 10 breast cancer families studied in detail, two harbour mutations in known breast cancer genes that were missed by clinical genetic testing either because the index case was a phenocopy who did not carry the mutation (BRCA2) or because the gene is not routinely tested in the context of breast cancer without additional clinical manifestations (PTEN). Among the remaining families candidate genes are currently being assessed for segregation.
among family members and for prevalence among an addition 800+ unexplained breast cancer families. In particular, we found truncating mutations in two genes that are involved in well-recognised DNA repair pathways but have not previously been associated with an increased risk of breast cancer. In summary, whole exome sequencing of multiple individuals from within each cancer family is proving to be an efficient strategy for rapidly identifying novel familial predisposing mutations.

**A42**

An audit of families with unreported or misreported cancers verified through a population-based cancer registry: implications for providing cancer risk assessment and management advice by a Familial Cancer Centre

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1) An unreported cancer in a family member is confirmed.
2) A reported cancer in a family member is confirmed for a different cancer site.

**Method:** Three hundred and fifty-six family pedigrees were submitted to the Cancer Council from Melbourne Health FCCs for cancer verification during 2010. Of these, 135 families had a one or more cancers confirmed where no cancer was reported and, 89 families had a different cancer confirmed to the one reported by the proband. Each of these pedigrees was reviewed, and data was collected on cancer risk assessment before and after the cancer verification information was obtained. For breast and ovarian cancer families, genetic testing eligibility scores were assessed using BRCAPRO. For bowel and uterine families, Immunohistochemistry (IHC) testing eligibility was assessed using the Revised Bethesda criteria.

**Outcomes:** This presentation will summarise data including cancer risk assessment, BRCAPRO scores, Immunohistochemistry eligibility before and after the cancer verification for a subgroup of families verified in 2010. This data will inform how the Cancer Council verification process will be presented, along with tools and methodologies and refine an implementation plan.

**References**


**A44**

Clinical practice improvement in the genetics clinic

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**Quality in health is defined as ‘doing the right thing, the first time, in the right way, and at the right time’. A model for practical quality improvement in the genetic unit will be presented, along with tools and techniques relevant to the practice of clinical genetics, and examples from real life practice.**

**Clinical Improvement follows five phases, including:**

- **Project Phase:** the process that needs improving is highlighted and defined, a team is formed, an aim or mission statement is produced, and a method of measurement is chosen.
- **Diagnosis Phase:** collect the evidence needed to diagnose the problem (its nature, its causes), and organise and prioritise the information using specific templates.
- **Intervention Phase:** involves using PDCA cycles to trial efficient methodologies and refine an implementation plan.
- **Impact Phase:** implement the changes, including implementation of a resistance management plan.
- **Sustaining Improvement phase:** develop plans for standardisation, documentation, measurement, review and training/education of staff.

In addition, preparation of results for submission to area health Quality Awards is reviewed, and preparation of results for publication.

**A45**

Is the whole greater than the sum of its parts?

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**Although mutations of BRCA2 are known to be associated with increased risks of prostate and pancreas cancer and melanoma, the prevalence of Jewish founder mutations of BRCA1 and BRCA2 (JFM’s) amongst**
incidental cases of these malignancies in Ashkenazi Jews has not been shown to justify founder mutation testing. This case report highlights the need to consider combinations of these cancers in Ashkenazi families.

John\(^a\) (pseudonym), aged 63, presented to the Hereditary Cancer Clinic at Prince of Wales Hospital. All four grandparents were Ashkenazi Jewish, and he became aware of BRCAl and BRCA2 JFM testing following the identification of the BRCA2 founder mutation in the husband of his sister Judy\(^b\). John’s brother had died from pancreas cancer at 60, his father had died from prostate cancer at 69 and his uncle had died from melanoma at 60. John was known to have an elevated PSA and was being treated for benign prostatic hypertrophy. There were no cases of breast or ovarian cancer in his family.

The combination of cancers was sufficiently concerning to offer John founder mutation testing. The 6174delT mutation of BRCA2 was identified. It was also identified in his sister Judy. Despite having a husband with the same mutation, she had three adult children with no evidence of Fanconi Anaemia. John’s mother was shown not to carry the mutation, so predictive testing was offered to his paternal relatives.

To date, 14 people in this family have had predictive testing and 5 unaffected carriers have been identified. In view of his mutation result, John underwent a further prostatic biopsy and cancer was found, resulting in a prostatectomy. Two unaffected relatives have had RRSO, one was shown to have dysplastic changes in the fallopian tube. Eligible tested males have been enrolled in the IMPACT study. Mutation carriers in this family aged between 40 and 80 have been offered participation in a trial of pancreatic cancer screening with endoscopic ultrasound.

Although this family had no cases of breast or ovarian cancers, it indicates that combinations of cancers associated with BRCA2 need to be considered for testing. Despite this being a large pedigree with information about the cancer status of all members, the paternal great grandparents of the proband had 38 descendent in the next 3 generations, of whom only 11 were female, contributing to the absence of any history of breast and ovarian cancer. Families such as this highlight the need to consider population JFM testing, which is being trialled in the UK as the GCaPPS (Genetic Cancer Prediction Through Population Screening) study, and being considered for Sydney through the POWH.

A46

From GWAS to genome sequencing: complementary approaches to identify melanoma predisposition genes

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A46

Family and twin studies indicate that melanoma susceptibility has a strong genetic component. Very rarely, melanoma runs in families in which there is an inherited mutation in a single ‘high penetrance’ gene, but in the general population melanoma susceptibility is thought to be governed by variation in a series of ‘low penetrance’ genes. We sought to identify new melanoma risk genes of both classes by conducting an Australian genome-wide association study (GWAS) of ~2200 melanoma cases and ~2200 matched controls (from the AMFS and G-MEGAS studies), in parallel with whole-genome sequencing of cases from densely affected melanoma families with follow up genotyping of interesting variants in the GWAS sample and other highly case-loaded melanoma families.

Genotyping of the GWAS sample was carried out using Illumina Hap610K or OMNI 1M arrays. All 25 SNPs that reached genome-wide statistical significance (i.e. p<5 x 10\(^{-8}\)) map to chromosomal regions/genes previously associated with melanoma (e.g. MC1R, ASIP, OCA2, MTA5/CDKN2A and SLCO4A2). However, two other genomic regions had multiple adjacent SNPs with low p values. These were the focus of a replication study using several melanoma GWAS data generated by groups from the MD Anderson Cancer Center and the International Melanoma Genetics Consortium (GenoME). Both independent GWAS data sets support the original Australian findings and thus indicate that the PARP1 gene and another broad region on 1q (which includes SETDB1, a recently identified melanoma oncogene) are novel low penetrance melanoma risk loci.

The three known high penetrance melanoma susceptibility genes (CDKN2A, CDK4 and ARF) account for less than half of all ‘familial’ melanoma. We sought to identify other genes responsible for susceptibility in multi-case melanoma families using a next-generation sequencing approach. Families were chosen on the basis that they did not have a mutation in CDKN2A, CDK4 or ARF; and had at least 5 melanoma cases. Whole-genome or exome sequencing was carried out on X cases from Y families. No convincing evidence has yet been obtained to support the identification of a new familial melanoma gene, however, follow up genotyping of several novel SNPs in the GWAS sample showed that a non-synonymous variant in the MITF gene is associated with melanoma in the general population. This type of integrated approach should help accelerate the discovery of new loci that play a role in the aetiology of melanoma.

A47

A genome-wide association study to identify genetic markers associated with endometrial cancer grade

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A47

Endometrial cancer is the most commonly diagnosed gynaecological cancer. Although endometrioid endometrial cancer (80% of cases) generally carries a good prognosis, some patients with this tumour subtype relapse within two years. Identifying genetic variants associated with prognosis could inform clinical decision-making for management at diagnosis, and inform development of chemotherapeutic agents targeting aggressive disease. Genome-wide association studies (GWAS) have been successful in identifying common genetic variation involved in cancer susceptibility. Presently there are limited published studies using GWAS data to identify single nucleotide polymorphisms (SNPs) associated with tumour prognostic indicators, such as grade. We used case data from an endometrial cancer case-control GWAS to assess association of SNPs with endometrial cancer grade. Genome-wide genotyping of 1285 Australian and British women with endometrioid endometrial cancer and reporting Caucasian ethnicity was performed using the Illumina 610K BeadChip. After applying quality control measures, data on 583,366 SNPs for 1220 cases with grade information were used in the analysis. PLINK software was used to assess SNP association with grade (p-trend<10\(^{-8}\)) have been selected for validation in independent sample sets. These SNPs are located in or near genes not previously reported to be involved in cancer aetiology or prognosis and, if confirmed, would represent novel gene targets. Neither of these SNPs fall within the top 1500 SNPs prioritised for validation of association with risk. Results to date suggest that genetic alleles associated with prognostic features, such as cancer grade, may be distinct from those associated with predisposition. GWAS analysis of tumour prognostic features is thus likely to improve understanding of biological pathways influencing outcome for endometrial cancer patients.
A48

'Next-generation' genome wide association studies
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A48

The first wave of cancer genome-wide association studies (GWAS) have revealed tens of independent loci marked by common variants of unknown or likely no functional significance that explain about 5-10% of familial risk for the particular disease. The approach taken to date has been conservative, and only a fraction of information has yet to be extracted from these expensive enterprises. For example, the Bonferroni procedure for selecting candidate phase II SNPs ignores many SNPs that happen to fail an extremely low p-value threshold. While this procedure does guarantee control of false positives, it seems counterintuitive to the purpose of phase I, which is to generate hypotheses based on promising candidates. Researchers have generally combined data from the discovery phase I and other phases and used 'genome-wide thresholds' based on assuming all SNPs are independent. Linkage disequilibrium (LD) makes it problematic to differentiate a real signal from highly correlated proxy signals. Most published GWAS do not examine SNP interactions due to: (a) the high computational complexity of computing p-values for the interaction terms, and (b) the typically low power to detect significant interactions. It is plausible that more information should be extracted if: (i) higher order interactions are fitted, (ii) highly selected cases and controls are used in phase I, (iii) large replication studies are used, especially if involving existing GWAS data, (iv) the non-independence of SNPs is taken into account using, e.g. BEAGLE CALL or haplotype analyses, (v) focus is on candidate gene pathways, and/or functional SNPs, and (vi) rarer and more SNPs, such as is available from the Illumina SM SNP chip, are used. We will illustrate these ideas using data from a GWAS of early-onset breast cancers, enriched for those with a family history, and a GWAS using extremes sample of extremes for mammographic density. We will also discuss the design of a large international breast cancer GWAS using the Illumina SM SNP chip, phase I cases enriched for family history, population-based phase II cases and controls, population-based family study of candidate SNPs, and GxG analyses using 'massively parallel' super computing.

A49

An audit of treatment focussed BRCA1/2 mutation testing at an integrated Familial Cancer Clinic
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A49

Background: The growth of a personalised approach to cancer treatment including surgical risk management strategies and Parp Inhibitor trials for BRCA1/2 mutation carriers has lead to the Peter MacCallum Familial Cancer Centre experiencing a rapidly increased demand for expedited risk assessment appointments +/- BRCA1/2 mutation testing. Expedited BRCA2 mutation screens are offered to individuals assessed eligible for publically funded testing and require these results in a defined time period to assist in their current cancer management.

The demand for expedited services places a burden on both the laboratory and clinic as patients need to be seen at short notice and have their genetic test take priority over routine tests.

Aim: To better understand the clinical utility and mutation detection rate of expedited BRCA1/2 tests, in order to improve services and resources for this group of patients.

Methods: A retrospective review of the Peter MacCallum Familial Cancer Centre’s BRCA1/2 mutation testing data from January 2007-April 2011 was performed. During this time period 119 patients were offered a treatment-focussed genetic test. The characteristics of the tested cohort were reviewed and analysed.

Results: Numbers of referrals for expedited risk assessment and requests for treatment focussed BRCA1/2 mutation testing remained consistent between 2007 and 2009 although have significantly increased in 2010 with nearly a twofold increase.

Most patients referred had been recently diagnosed with breast cancer and were considering the option of breast conservation versus mastectomy. To assist them with this decision they were seeking advice about their genetic risk of developing a new primary breast cancer. In addition, referrals were also received to determine the eligibility of some patients for Parp Inhibitor trials.

A pathogenic mutation was detected in 20/119 (16.8per cent) of patients who had an expedited BRCA1/2 test. Further analysis about the pre and post testing treatment decisions is being analysed and will be presented.

Conclusion: In 2010 a significant increase in demand for treatment focussed risk assessments and expedited BRCA1/2 tests was experienced by the Peter MacCallum Familial Cancer Centre. Further data will be presented on the characteristics of these cohorts and the clinical utility of expedited assessments.

A50

Audit of adherence to GI screening recommendations for Lynch Syndrome Patients
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A50

There is clear evidence documenting the morbidity and mortality benefit of regular colonoscopy surveillance in reducing the high risk of primary and metachronous colorectal cancers associated with Lynch Syndrome (LS) (Jarvinen et al. 2000). The Australian NHMRC guidelines (2005) recommend LS patients undergo a colonoscopy every 1-2 years (NHMRC 2005). At Peter Mac yearly colonoscopy screening is usually advised for LS patients.

The long term adherence rate to colonoscopy in LS patients as reported in the literature is between 60-88% (Stoffel et al 2010, Wagner et al 2005). Some factors have been associated with inadequate LS patient screening adherence including lack of sedation, inappropriate advice from managing doctors, financial costs, embarrassment and lack of patient time (Bleiker et al 2005). Whilst not studied, some of these factors may also contribute to a phenomenon known as ‘screening fatigue’, whereby after one or more normal screening procedures patients begin to attend less frequently for the recommended screening procedures. At the Peter Mac patients and their managing doctors are given GI screening recommendations by the FCC when the patient receives their gene mutation results. However, it is unclear whether LS patients continue to follow the GI screening advice set out by an FCC over time and whether there is any evidence that ‘screening fatigue’ exists. Furthermore it is unknown whether differences regarding the frequency of colonoscopy offered/ performed, adherence to colonoscopy or quality of endoscopy exist for patients who participate in a high risk GI management clinic versus those who are screened by endoscopists in generalist community based settings.

The FCC at Peter Mac undertook an audit of the GI screening practices of 74 confirmed Lynch Syndrome gene mutation carrier patients from 2006-2010. Of these patients 27 participate in a high risk GI management clinic where they receive their GI screening. The additional 47 patients have GI screening conducted through either private endoscopy clinics or at public hospitals.

This work will:
❖ Provide a clinical description of our population of LS patients
❖ Describe the rate of adherence to recommended GI screening timeframe. This is assessed by comparing the screening practices of LS patients with the recommendations provided by the FCC at the time of their gene test result. We will also present data about whether updates of screening recommendations by our FCC were acted upon by patients/ their managing doctors.
❖ Assess for evidence of ‘screening fatigue’
❖ Compare rates of adherence to GI screening practices between patients attending GI high risk management clinics versus patients attending for GI screening in community settings.
A51

Observation of the cancer patient journey: a learning curve for Genetic Counsellors
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):AS1

Cancer genetic counsellors typically work in hospitals and participate in multidisciplinary clinical teams. However, in their training, genetic counsellors seldom have much exposure to the medical environments in which their clients receive their cancer treatment. Although there is a general understanding about screening, learning a diagnosis, surgical, chemotherapy, and radiotherapy treatments, many of the genetic counsellors have no first hand experience of these processes, making it difficult to comprehend the impact it has on clients.

This project aimed to develop knowledge and awareness of what a cancer patient would experience throughout their cancer journey, therefore increasing the scope of empathy and consideration for clients seen. Secondly, this project aims for genetic counsellors to gain a better appreciation of the risk management procedures and surgeries that enter into the genetic counsellor’s discussions with clients of a Familial Cancer Centre (FCC).

Two genetic counsellors approached several senior clinicians (including Medical Oncologists, Gastroenterologists, Surgeons, and Gynaecologists) affiliated with the Royal Melbourne Hospital FCC to observe both consultations and medical procedures in their specialty area.

The genetic counsellors reflected on their observations of the patient’s cancer journey and how the patient’s experiences may impact on a genetic counselling session. Both counsellors gained a greater insight into the enormity of a cancer diagnosis on an individual.

We wish to share our experience: a greater knowledge of the screening and surgical recommendations that are discussed in a genetic counselling session, an increased awareness of the role of other medical professionals in the patient’s cancer journey and a better understanding of how the FCC integrates with the patient’s cancer journey. This project warrants extension as we believe that by being better equipped to understand our patients and their needs, we can provide better patient care.

A52

Use of unit standard paragraphs and letters in: 1. Australian Familial Cancer Centres 2. One Centre’s experience at Southern Health
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):AS2

Dictating and checking of letters is a time consuming but essential part of the work of all Familial Cancer Services/Cancer Genetics units. 1. We contacted Familial Cancer Centres/Clinical Genetics units across Australia (17 in total) to ask about the use of unit standard letters and paragraphs for Familial Cancer patients in each service. - 14 services provided data. Both Genetic Counsellors and unit typists were contacted where possible:

- 5/14 services had both unit standard letters and paragraphs.
- 3/14 services had neither unit standard letters nor paragraphs.
- 6/14 services had one or the other.
- 5 units had no unit typist although some doctor’s typing was outsourced.

Unit standard letters were used more often than standard paragraphs. These standard letters were regularly used by Genetic Counsellors in the majority of practices across Australia, and infrequently used by unit doctors. However in 3 services, standard letters were used by most staff.

The use of standard letters was generally thought to be efficient and led to consistency of information across a department but problems included:
- Modifications to the standard template, which typists found reduced time savings;
- Lack of personalisation of information;
- Poor flow of the letter.

2. Our service has standard paragraphs, but these have not been regularly used. In early 2011 we undertook reformatting (and implementing) standard letters and paragraphs for breast/ovarian patients (80% of our service). Three of our clinic staff (2 genetic counsellors, 1 doctor), who had not previously used standard letters or paragraphs implemented using these reformatted changes with the aim of reducing clinical time checking letters and particularly in our unit, to reduce the typist’s work.

Prior to use of the standard letters/paragraphs, it was estimated the typist took 17 minutes (n=30 letters) to type a patient letter and 10 minutes to type doctor letter (n=19 letters). It took the 3 clinicians an average of 12 minutes (n=36 letters) to check a patient letter and 6 minutes to check a doctor letter (n=28 letters).

Early data of standard paragraphs shows little change in typing time, but some drop in clinician letter checking time. Multiple modifications made to the standard letter template are thought to be the main barrier to a reduction in typing time. We continue to review our use of standard letter templates.

A53

Making a good model better - evaluation of the NSW Combined Family Cancer Clinic meetings
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):AS3

Ensuring best practice in the NSW/ACT Family Cancer Service has considerable challenges given the geographical dispersion of geneticists, oncologists and genetic counsellors and the small often isolated teams in which they operate. The combined monthly meetings of 25-30 genetic health professionals were first initiated by a senior geneticist in partnership with the Centre for Genetics Education in 1996 in order to share expertise, keep abreast of rapidly developing evidence, and provide a network of support. The 3 hour meetings have been well attended, with four meetings per year via video/ teleconference. A previous evaluation was conducted in 2004. New IT technologies are providing flexibility in conferencing and an increased demand on genetic services means time away from the workplace for meetings may be less cost effective. Consequently a recent evaluation was undertaken to identify the perceived outcomes of the meeting and inform future planning and funding.

A questionnaire requesting anonymous participant views on the current format, impact of the meetings on clinical practice, preferred mode of meeting and ideas for future development was distributed. 22/25 (88%) surveys were completed (though 2 were invalid). Overall, everyone was satisfied with the meeting experience and format with 90% very to extremely satisfied. 85% of participants agreed or agreed strongly that the meetings impacted positively on their clinical practice. Perceived outcomes of the meeting included: expert opinion gained on complex cases; successful avenue for updating knowledge of current literature; engagement with relevant literature through journal presentations; enhancement of clinical practice through case presentation and discussion and enhancement of patient outcomes through networking. Despite the opportunities available through IT, participants reported the inconsistency in delivery of videoconferences and the preference for face-to-face meetings. Participant comments included: ‘This is a brilliant meeting that is well worth attending each month.;’ ‘Excellent opportunity to review difficult cases & go back to a patient/family with confidence.;’ ‘I think these meetings help cohesion in cancer genetics - uniformity.’ The perceived impact on clinical practice by participants and the high level of satisfaction is a strong endorsement for the continuation of the FCC meetings.
What are the unmet support needs of women with a known BRCA1/2 mutation?

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A54

Background: Women known to carry a BRCA1/2 mutation have to make complex decisions regarding the management of their increased cancer risk, such as whether or not to undergo risk-reducing surgery and how to communicate this risk information to family members. These women may face a unique set of supportive care needs, although unlike the situation for breast and other cancer patients, few supportive care services for mutation carriers exist outside the Familial Cancer Centres. The aim of this presentation is to describe the unmet support needs of women with a known BRCA 1/2 mutation volunteering for a randomised control trial (RCT) testing the effectiveness of a telephone based peer support program.

Methods: Participants were recruited through Familial Cancer Centres in Victoria, New South Wales and South Australia. Women between the ages of 18-70, who tested positive for a BRCA1/2 mutation within the last 4 years were eligible for participation in the study. Participants were recruited as part of an ongoing RCT. Women completed a baseline survey after enrolling in the study and prior to randomisation. The baseline survey included a 16-item scale assessing unmet support needs, such as dealing with fears about developing cancer, wanting reassurance that feelings are normal, and talking to others in a similar situation. Women were asked to indicate their level of need in the past month on each item using a 5-point likert scale.

Results: 216 participants have completed the baseline survey to date (response rate of 46%). The average age of participants was 47.2 years (SD:13.6) and 44.0% (n=95) had a previous diagnosis of breast or ovarian cancer. The mean time since notification of carrier status was 2.5 years (SD:2.1).

The average number of moderate to very high needs among all participants was 4.7 (S.D 4.6). Twenty six percent (n=52) of participants had no moderate to very high needs, while 23.1% (n=46) had a moderate to very high need on eight or more items. The most commonly endorsed moderate to very high needs were: “dealing with uncertainty about the future” (42%), “dealing with the impact a faulty gene has had on your family” (40%), and “dealing with fears about developing cancer” (39%). The least endorsed items were: “dealing with insurance issues” (61% indicated no need), “dealing with feelings of isolation” (53% no need) and “obtaining more information about your risk for breast cancer” (53%).

The average number of moderate to very high unmet needs was not related to previous history of cancer (P=0.94), having children (p=0.87) or time since receiving BRCA1/2 mutation status results (P=0.36).

Discussion: Many women with a known BRCA 1/2 mutation have unmet supportive care needs that are not currently being addressed. Level of unmet need is not related to time since receiving test results, history of cancer or having children. The RCT trial will assess whether a telephone-based peer support program meets these unmet needs.

In 2009 the successful applicant for the genetic counsellor position working predominantly with this clinician was of Muslim faith, apparent in her dress. Based on the tensions felt between these two minority groups worldwide, the team were concerned that this may be a barrier to good communication and may result in distress to some Jewish patients. The team consulted Area Employment Services Unit, and it was decided to discuss their concerns with the new counsellor and provide her with an opportunity to resolve any concerns she may have. Together they decided that there would be no changes to standard work practices, but that both the clinician and the genetic counsellor would be alert to any discomfort by the patients and see if there was any negative feedback from them.

54 intakes of Jewish patients by the genetic counsellor via telephone or face to face were done in one year, with patient age ranging from 23-67 years. Excellent rapport was built over the telephone with the patients. Most patients shared personal stories openly (the only issue being time management). With face to face consults, the conversation often started with discussion about the scarf worn by the counsellor, which then moved to the real purpose of the consultation. Most patients were aware that they were to be seen by a Jewish clinician. It was noted that none of the 54 Jewish patients expressed the desire to be seen solely by the Jewish clinician. No patient expressed concern about being seen by a Muslim counsellor to either the clinician, other team members or hospital services. Several patients required in-depth counselling with the genetic counsellor, leading to five referrals to the oncology psychologist. Their complete disclosure was evidence of the trust they felt in the genetic counsellor. Follow-up phone calls to patients after the clinic appointment were universally pleasant with patients expressing satisfaction with service provided, and often making extremely positive remarks about both the clinician and genetic counsellor. A number openly commented on the excellent service provided by the Muslim counsellor.

Conclusion: Several factors were key to the success of this cross cultural service provision:

• Discussing the potential for patient distress before employment began, so both staff members felt comfortable to maintain a dialogue on the issue
• Underlying excellent rapport between the clinician and genetic counsellor
• Good communication skills of the genetic counsellor to establish good rapport with the patients
• Open discussion about the Muslim faith of the counsellor with patients when appropriate
• The needs of patients for genetic services were met by the team.

Improving the provision of Melbourne Health Familial Cancer services to Victoria’s Culturally and Linguistically Diverse (CALD) communities

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A56

Heritable cancer syndromes affect all ethnicities, however, people from culturally and linguistically diverse (CALD) backgrounds do not attend the Royal Melbourne Hospital (RMH) Familial Cancer Centre (FCC) in the numbers that reflect the diversity of the Victorian population. Despite interpreters routinely organised, there still remains an impediment to attendance. Based on this observation, we considered the possible barriers preventing participation by CALD communities at the FCC, and developed initiatives to target these barriers.

Our first venture in trying to answer this question involved participation in the inaugural “Cancer Awareness Expo” in October 2010 organised by the Chinese Cancer Society of Victoria (CCSV). In preparation, we translated and printed the FCC brochure into traditional and simplified Chinese (suitable for Mandarin and Cantonese speakers, respectively) and these were available to the participants. Three RMH genetic counsellors were available throughout the day to engage with the participants with the assistance of interpreters. We learnt that, for this community, one of the main barriers to participation in the FCC is lack of awareness of our services.
We surmised that there would be other non-English speaking communities in Victoria for which language may limit awareness of our services. Therefore, we undertook to translate and print our FCC brochure into nine additional languages, namely Arabic, Croatian, Greek, Italian, Serbian, Somali, Spanish, Turkish and Vietnamese. We targeted these ten languages based on interpreter-use at the three hospitals the FCC services. We also established, using data collected by the Cancer Council Victoria, that people from these language backgrounds, are affected by breast and bowel cancer[1] – two of the cancer types that can be associated with an inherited predisposition.

As far as we are aware, this initiative, of producing an FCC brochure in languages other than English, is a first within Australia.

We have since taken further initiatives to raise awareness to CALD communities. We are involved with the “Living with Cancer” programs for Italian and Greek communities and are looking into establishing connections with other community groups. Our translated brochures have been sent to General Practitioners that speak one of these ten targeted languages and distributed amongst the oncology clinics within the hospitals that the RMH FCC services.

We believe that these ongoing initiatives are steps towards providing a more culturally-appropriate and equitable healthcare service to members of the CALD community in Victoria. We plan on continuing to engage with CALD communities, and evaluate the impact that this has had on the number of patients who engage with the RMH FCC in the future.

Reference

A57
Case study: positive outcomes from a negative
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A57

Background: As the work load for clinical genetics escalates and more genetic test are ordered, the potential for errors increase. This report present at the affected patient’s request, the occurrence of an error and its subsequent management.

A BRCA2 large deletion had been detected in a family member and predictive testing had already occurred in other family members. Our client had 50/50 chance of having the mutation and had a negative predictive test. When breast cancer occurred in the patient, the sample was retested and found to be positive. The different results were given in person and a root cause analysis done. The patient requested that the error be discussed at clinical meeting and so lessons found could be learnt by the whole genetics community.

Multiple factors impacted on this case. An unclassified variant-considered likely to be benign, had been identified, as well as a pathogenic mutation. Blood was collected from both relatives one day apart and sent for testing at the laboratory which identified the mutation. Although the sample request form requested a predictive test specifying the gene, lab ID and DOB of the proband it did not specify the mutation to avoid transcription errors. A new laboratory staff member incorrectly tested for the unclassified variant. Although duplicate testing was done the samples were not collected independently and the same error occurred. The clinical staff were rushed as there were 2 carriers in the breast clinic, one a newly diagnosed affected by breast cancer and another a possible diagnosis, requesting consultations.

A comprehensive review of the clinic’s genetic testing protocol has tightened protocol to minimize future error; the request form is accompanied by a de-identified copy of the mutation even if the testing laboratory did the mutation search. Although the protocol required predictive test results to be checked against the proband’s result will be done by the doctor and counselor before being given. Cost consideration are ongoing as to whether the second sample will be sent completely separately. Errors are being made to prevent the clash with the breast clinic.

Errors in genetic testing are rare event and give the opportunity to review procedures. Knowledge of errors allow other clinicians to review their protocols. We have learnt not only from our errors but from the valuable input from other clinicians who have shared their traumatic experiences. We propose that documentation of the extent and cause of genetic testing errors occur at the family cancer clinic day next year or at the COSA meeting. A culture of open disclosure with colleagues as well as the clients affected will help guard against further avoidable errors and help us develop sustainable, attainable and cost effective processes.
with overexpression associated with more aggressive clinical course and chemo-resistance. In vitro cell lines and tumour xenografts of prostate cancers have shown overexpression of RAD21 compared with benign epithelium. Located at 8q24, this also appears to be a region often amplified in aggressive prostate cancer in vivo. We examined for RAD21 expression in familial prostate cancer.

Methods: TMAs were created from 40 prostate cancer samples including 11 BRCA2 and 13 BRCA1 associated tumours. IHC staining for RAD21 was correlated with E-cadherin, β-catenin, Androgen Receptor (AR), MUC1, AMACAR, Cyclin-D1 and clinico-histological factors.

Results: 33% of tumours overexpressed RAD21, with a higher proportion of BRCA-X tumours (80%, p=0.0082) compared with BRCA2 and sporadic cancers. RAD21 was positively correlated with Ki-67 (p=0.0053) and inversely with aberrant E-cadherin expression (p=0.0323). Within the sporadic/non-BRCA1 tumour group, there was positive correlation with AR (0.0070) and Cyclin-D1 (p=0.0270).

Conclusion: RAD21 expression is commonly present in prostate carcinoma and may be particularly important in the pathogenesis of BRCA1 associated tumours. Within sporadic non-BRCA1 associated tumours, there is an association between RAD21 and AR expression, which may be biologically relevant and distinct in pathogenesis from those with aberrant E-cadherin expression.

A60
Genetic testing and immunohistochemistry for SDHB in phaeochromocytoma-paraganglioma syndromes: the South Australian experience
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Aim and methods: a retrospective review of germline genetic testing (VHL, RET and succinate dehydrogenase subunit genes SDHB, SDHC and SDHD) and immunohistochemical staining for SDHB in tumour tissue (SDHB-IHC), in patients referred to the South Australian Familial Cancer Unit with an adrenal phaeochromocytoma (PC) and/or paraganglioma (PGL).

Results: between January 1999 and May 2011, 24 probands were referred to and assessed by our service. The clinical presentation and mutation pick up are presented in the Table. Tumour tissue was available from 20 probands and SDHB-IHC was abnormal in all probands with an SDH mutation (5/5; 100%), 0/1 with a VHL mutation and 2/12 (16%) with no identified mutation (the 2 probands with abnormal SDHB-IHC both presented with familial head & neck PGL). Tissue was unavailable for testing in the remaining 4 patients; 3 with a RET mutation and a MEN2 phenotype; 1 with an SDHD mutation and familial head & neck PGL (SDH-IHC is pending in her affected sister). Table 1.

Conclusion: our experience supports using SDHB-IHC as a tool to triage genetic testing in patients with PC or PGL.

Reference

A61
Pathogenic germline TP53 mutations in adult sarcoma patients; implications for treatment and screening – description of an upcoming project
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Identification and clinical annotation of risk alleles is critical for young people with sarcoma for several reasons. The mutated gene can be passed onto the next generation affecting reproductive choices. In addition, the risk of a second malignancy appears to be markedly increased in some patients. Thirdly, the risk to other relatives, parents and siblings may vary from 50% to an unquantifiable risk. Finally, ionizing radiation increases cancer risk synergistically in the presence of mutations in sarcoma genes such as RB and TP53 and diagnostic and therapeutic radiation is modifiable in high risk individuals provided such mutations are identified.

The primary aim of this project will be seeking to establish whether the detection of pathogenic germline TP53 mutations in adult sarcoma patients can improve outcomes through tailored clinical management. These patients have been recruited through the International Sarcoma Kindred Study (ISKS). This project will have access to the ISKS biospecimens, pedigree data, genetic information and data associated with clinical outcomes. The ISKS currently has 450 adult sarcoma probands, with recruitment on going. To date, sequencing has already been performed on 272 of these probands, in which 12 pathogenic mutations have been detected (4%).

Index cases with an identified pathogenic TP53 mutation and their families will be systematically followed up to ascertain outcomes and to recruit further family members. The genetic status of this cohort will be collated with their clinical characteristics through examination of medical records and family history through pedigree data. The clinical outcomes of the identified pathogenic TP53 mutation carriers and treatments given will be documented and compared against affected non-mutation carriers using a pseudo case-control study design to assess how the effects of treatment combine with mutation status.

A further aim of this project will be to conduct analyses nested within the whole ISKS cohort to seek to establish the pathogenicity of unclassified variants that have been detected so far in the ISKS cohort.

Table 1(abstract A60)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No.</th>
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<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Apparently sporadic unilateral PC</td>
<td>10^*</td>
<td>1</td>
</tr>
<tr>
<td>Familial PC</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PC &amp; MEN2 features</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Apparently sporadic head &amp; neck PGL</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Familial head &amp; neck PGL</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Apparently sporadic malignant abdominal PGL</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>24</td>
<td>10</td>
</tr>
</tbody>
</table>

* presented with unilateral PC and developed a head and neck PGL 14 years after the PC
^ VHL and RET testing incomplete in 2 (complete testing will be presented at the meeting)
This analysis will be twofold with the first part of the analysis being conducted with novel variants that have not yet been previously described. Once these variants are classified using an integrated evaluation approach, they will be examined alongside other variants detected in the cohort that have previously been described but where the magnitude of their effect on the risk of sarcoma has not yet been defined. A case-control study will then be conducted, which will be seeking to establish whether these variants are found at a higher frequency in sarcoma patients than the general population, thus implying an association with sarcoma. Based on this information, these variants will then be graded according to the guidelines recommended by the IARC Unclassified Genetic Variants Working Group to guide clinical management for the different variants.

A62

Chemoprevention with the metabolism modifying drugs dichloroacetate and metformin in Trp53+/− mice
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A62

While genetic testing for familial cancer susceptibility factors has excelled in recent years, the prevention options for those carrying high risk alleles have not. Altered bioenergetics has now been acknowledged as an emerging hallmark of cancer, and is believed to be a critical characteristic acquired during tumorigenesis. Several very safe drugs are available that can modify bioenergetics. Dichloroacetate (DCA) inactivates pyruvate dehydrogenase kinase, resulting in activation of pyruvate dehydrogenase, reduced lactic acid production and increased mitochondrial activity.

Metformin, a type 2 diabetes treatment which activates AMPK, thereby inhibiting mTOR, has unambiguously been demonstrated to reduce the risk of many cancer types in diabetics where insulin treatments do not. We have tested these drugs as chemopreventive agents against the mammary tumours that occur in the spontaneous BALB/c-Trp53+/− mouse tumour model.

In vitro studies on breast cancer cell lines indicate that DCA (1-5 mM), metformin (30-300 uM) or the combination of the two can significantly inhibit breast cancer cell growth over 4 days of treatment. These results support the possibility that DCA and metformin could prevent or delay breast cancer formation in BALB/c-Trp53+/− mice. To examine this, four groups of female BALB/c-Trp53+/− mice were given distilled water (n=75), DCA (1.5 g/L in drinking water, ~180 mg/kg/day, n=53), metformin (0.25 g/L in drinking water, ~30 mg/kg/day, n=61) or DCA + metformin (n=51) from 8 weeks of age, and monitored for tumour development over 78 weeks. The overall tumour-free survival curves were not significantly different (Kaplan-Meier analysis) between the treatment groups, suggesting that metformin does not reduce cancer risk in non-diabetics. However, the occurrence of mammary tumours in the four groups was altered. DCA reduced the number and increased the latency of mammary tumours (20.8% of DCA treated mice with mean latency of 63.8 weeks compared to 28.0% of untreated mice with mean latency of 55.0 weeks), whereas metformin had no effect (26.2% of mice, average latency 54.7 weeks).

Examining the mammary tumour-free survival curves, DCA appeared to eliminate the early onset mammary tumours (latency <52 weeks, p=0.02), while not affecting the occurrence of longer latency tumours. We are currently examining these tumours to identify characteristics that may explain this difference in sensitivity to DCA prevention. Contrasting with the in vitro cancer cell line result, the two drug combination had worse outcomes for tumour development, with 35.3% of mice developing mammary tumours with a decreased latency of 48.8 weeks (p<0.02 compared to DCA alone). Preliminary western blotting results in MDA-MB-468 breast cancer cells found that DCA could block the activation of AMPK by metformin, indicating the potential for drug interactions. However, the outcomes of these drug interactions clearly differ in the fully transformed cancer cell (growth inhibition) compared to the preneoplastic cell (survival / growth advantage) and requires further investigation.

Acknowledgements: This research was supported by NHMRC 366787 R. D. Wright Career Development Award, a National Breast Cancer Foundation Novel Concept Award, and Cancer Australia PCDCRS.

A63

Referral of Queensland women with endometrial cancer to genetic services
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A63

Approximately 5% of all endometrial cancers are due to a hereditary disposition, and a majority of the cases were found in families with Lynch syndrome or hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Whilst both men and women with Lynch syndrome have a significantly increased risk of developing colorectal cancer (18-69%), women face the additional lifetime risks of developing endometrial cancer – between 27% to 71% as compared to 2% in the Australian general population. Despite the increased risk, many eligible women who may benefit from genetic assessment are not being referred by their treating clinician. The purpose of this study is to evaluate the patterns of referral of women diagnosed with endometrial cancer to genetic services. Using the diagnostic, clinical and referral databases from three different sites, we were able to link data of endometrial cancer cases in Queensland from May 2005 to December 2007. We determined the percentage of women diagnosed with endometrial cancer who could have been referred based on at least one risk factor suggestive of Lynch syndrome, the percentage of women that were referred and the percentage of women that attended genetic services. The revised Amsterdam and Bethesda criteria guidelines were adapted and used to assess the appropriateness of referral. Preliminary results show that of the 955 new diagnosis of endometrial cancer, 29 women (3%) were referred and 17 (1.8%) attended. This suggests that women who may benefit from genetic assessment do not ultimately attend their scheduled appointment. The mean age of referral is 61.7 years, with seven women diagnosed under the age of 50. Of the seven women, three were found to be mutation carrier. The results will be used to improve identification and referral of women at risk of Lynch syndrome to genetic health services in Queensland, and to increase awareness of hereditary gynaecological cancer.

A64

Familial platelet disorders with a predisposition to acute myelogenous leukaemia: a RUNX1 update
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A64

Background: Familial platelet disorder with a predisposition to acute myelogenous leukaemia (FPD-AML, omim#601399) is an autosomal dominant disorder that is linked to mutations within the RUNX1 gene. The RUNX1 gene, present on 21q22.1, plays a role as a regulatory switch in both embryonic and adult haemopoietic development. Heterozygous mutations in RUNX1 are a common feature in FPD-AML with different prognostic outcomes reported to be attributable to the location of the mutation within the protein domains.

The Australian Familial Haematological Cancer Study (AFHCS) currently has 55 Australian families registered with predispositions to haematological malignancy. RUNX1 mutations have been found in 11 patients from 3
families diagnosed with AML. This figure is predicted to be higher if screening occurred when FPD was first detected.

The IMVS, Adelaide, now offers full RUNX1 gene screening. To date, we have screened 25 individuals and confirmed germline mutations in 2 AFHCS families. We have reported a novel germline heterozygous nonsense mutation (c.958C>T, p.Arg320X), and a deletion of exons 2, 3 and 4 (c.59-32857_508+2502del).

Recent research is highlighting the role of monoallelic RUNX1 mutations in the generation and progression of pre-leukaemic FPD to AML. The evidence suggests that different prognostic outcomes are dependent on the impact the mutation has on the final product, although there is a wide degree of genetic heterogeneity observed. This has implications for the management and treatment options available to individuals affected. It has also proved useful in selecting family members negative for the familial mutation who may be suitable as bone marrow donors.

**A65**

When, how and why BRCA1 and BRCA2 genetic testing is offered to patients who do not meet standard criteria

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*Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A65*

Various BRCA1 and BRCA2 mutation probability prediction models exist which can be used to determine equitable access to publicly funded genetic testing. In January 2009 two of these models were introduced in three New South Wales genetic services. From this time, the criteria below were used to determine eligibility for publicly funded BRCA1 and BRCA2 mutation analysis.

- Individual diagnosed with breast or ovarian cancer with one or more of the following:
  - Combined Manchester score of 15 or higher
  - Combined BOADICEA score of 10% or higher
  - Synchronous or metachronous breast and ovarian cancer
  - Serous fallopian tube cancer

Cases not fulfilling the above criteria could be offered testing following discussion and approval at a combined monthly meeting of clinical staff. This was instigated because staff thought that some clinical scenarios would not easily fit into the BOADICEA or Manchester models and yet should be considered for testing.

We have audited all 77 cases discussed at the meetings between January 2009 and December 2010. Some cases discussed did in fact meet the testing criteria above. In 41 cases the decision was made to offer publicly funded BRCA1 and BRCA2 mutation analysis.

Reasons cases were considered for ‘off-criteria’ testing and bought to the meeting for discussion included:

- Very early age of diagnosis
- Pathological subtype
- Limited family structure
- Non-Western European ancestry
- Affected with prostate or pancreatic cancer
- Ashkenazi Jewish ancestry but no BRCA1 or BRCA2 founder mutation identified

We will present an analysis of the 77 cases including case and family history, pathology, BOADICEA score, Manchester score, outcome of discussion, and genetic test results. We will also consider the cost-effectiveness of ‘off-criteria’ testing.

**A66**

Validation study of risk prediction models for female relatives of Australian women with breast cancer

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*Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A66*

Risk prediction algorithms are an important tool for identifying individuals at high risk of developing the disease who can then be offered individually tailored clinical management. Several algorithms that predict the probability of breast cancer incidence are currently used in clinical practice. It is uncertain, though, as to which of the breast cancer risk prediction models performs best for female relatives of Australian women with breast cancer.

We evaluated the performance of the risk prediction algorithms BOADICEA, BRCAPRO and the Gail model using 879 families of ABCFS case probands, half of whom were diagnosed before age 40 years and the remainder before age 60 years. Cumulative breast cancer risks over 10 years of follow-up were calculated for 2000 unaffected female relatives. A total of 93 incident breast cancers were reported. The ratios (95% CI) of expected to observed number of breast cancers were 0.69 (0.56-0.84) using BOADICEA, 0.62 (0.51-0.77) using BRCAPRO, and 0.89 (0.73-1.09) using the Gail model. Tests for discrimination (ROC curve) were BOADICEA=0.61, BRCAPRO=0.64, and the Gail model=0.65.

Similar analyses using other models, including the Tyrer-Cuzick (IBIS) and Claus models, will also be presented.

**A67**

PREMM 1,2,6 MODEL1 as a new gene specific prediction model for Lynch Syndrome: retrospective review of mutation positive cases

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*Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A67*

Lynch Syndrome, also called Hereditary Nonpolyposis Colorectal Cancer (HNPPC), occurs as a consequence of germline mutations in the mismatch repair (MMR) system (Stoffel et al., 2009), mainly in MLH1 and MSH2 (>90% of cases), but also in MSH6 and PMS2 [1]. Kastrinos et al., (2011) developed a new web-based multivariable polytomous logistic regression model for Providing gene specific Risk Estimates (Model) (PREMM 1,2,6) that uses genotype-phenotype data from MLH1, MSH2, and MSH6 mutation carriers to generate individualized predictions for each gene based on personal and family history characteristics [1] recommends that a gene prediction of >5% should be a threshold for a referral for further genetic risk assessment.

A search was performed to obtain a list of patients seen at the Prince of Wales Hereditary Cancer Clinic who were sequenced for mutations in MLH1, MSH2 or MSH6 from 1994 to 2011 with 78% occurring after 2005. A total of 40 patient pedigrees were obtained and used for the PREMM 1,2,6 model, 29 were mutation positive and 11 were tested inconclusive. 17% of probands were selected for testing based on tumour testing and the remainder were selected based on family history alone. The model included proband and first and second degree relatives age and diagnosis of colorectal cancer and endometrial cancer. This model also used an inclusion for extra colonic Lynch-associated cancers. Each proband was also assessed by the Amsterdam II criteria and given a risk category according to the Australian Cancer Network Guidelines of average, 1, 2 or 3.

A gene-specific mutation prediction cut off of 5% was instigative of a mutation for 21 probands (8/12 for MLH1, missing 33%, 11/13 for MSH2, missing 2% and 2/4 for MSH6). Based on this limited data, endometrial cancer was highly predictive for MSH6. The prevalence of extracolonic, nonendometrial HNPPC-associated cancers was higher among MSH2 carriers than MLH1 and MSH6 carriers, being weakly predictive. The specific gene estimates were not useful for when tumour analysis was unavailable, with 59% (17) mutation positive probands having a low PREMM1,2,6 score, which would not have support a decision to pursue single gene testing.

Amsterdam II criteria and Australian Network Guidelines have been previously found to be an ineffective strategy for triage for gene testing for Lynch Syndrome. Further study on a larger cohort is needed to determine if the PREMM 1,2,6 model is an effective triage tool for gene testing for Lynch Syndrome.

Reference

A68

Searching for BRCA3 by exome sequencing
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2)A68

The current paradigm suggests that some non-BRCA1/2 multiple case breast cancer families are caused by rare mutations in high-risk genes. We are using exome sequencing to identify potential BRCA3 genes in a small number of KConFab families. We selected five non-BRCA1/2 families containing 5-9 breast cancer cases (of which 0-4 per family were affected under the age of 40) who have had extensive analysis of BRCA1 and BRCA2, and for which germline DNA was available from two distant relatives (at least 4th degree) affected with breast cancer between 30 and 56 years of age. In first stage we isolated the exomes of two pairs of affected individuals (4th and 5th degree relatives, from two families) diagnosed with breast cancer between the age of 30 and 39, using the SeqCap EZ exome capture kit ( NimbleGen) and then sequenced the libraries on the GAIIx and HiSeq (Illumina) platforms with a paired-end protocol. The sequences were aligned to the human genome with BWA and we then compared several pipelines for processing the sequences and calling variants. The best results were obtained with the Picard/GATK pipeline after local re-alignment and re-calibration of the base quality. The samples from the remaining three families will be sequenced by Axeq Inc. and the results of the analysis will be presented.

A69

Bowel cancer in a 17 year old: what could be the reason?
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2)A69

Inherited conditions predisposing to early onset bowel cancer include the autosomal dominant conditions of Lynch Syndrome, Familial Adenomatous Polyposis and PTEN Hamartoma Tumor Syndrome, as well as the autosomal recessive condition of MUTYH-Associated Polyposis. There is also an increased risk of early onset bowel cancer and other malignancies in individuals with bialleic mutations (homozygous or compound heterozygous) in the mismatch repair (MMR) genes. Screening recommendations are usually given to family members on the basis of the clinical diagnosis assisted by germline genetic testing for the proband and at-risk relatives. For an individual with very early onset bowel cancer, careful consideration of the pathology and wider attention to phenotypic features and/or their family history provide clues as to whether there may be an underlying genetic predisposition. These include: age at diagnosis; clinical examination; presence or absence of polyps; results of immunohistochemistry (IHC) for the MMR gene proteins +/- BRAF testing; tumour microsatellite instability (MSI) testing, and family history of bowel cancer or other syndrome related cancers. Sometimes, no clear conclusions can be reached about an underlying inherited mechanism, if any. We will present the case of BK, who was diagnosed with caecal cancer at the age of 17. A right hemicolectomy demonstrated a moderately to poorly differentiated adenocarcinoma, with a 40% mucinous component and tumour infiltrating lymphocytes. No precursor lesion was identified, and no polyps were identified in the distal bowel or in the resected bowel. Three lymph nodes out of 32 were positive for adenocarcinoma. Staging was negative for distant metastatic disease. Adjuvant chemotherapy with FOLFOX was given. BK's parents are second cousins. The only family history of cancer was a fifth-degree relative with bowel cancer. The results of genetic investigations will be presented, along with a reflection on the challenges for setting screening recommendations when two potentially at-risk relatives are severely intellectually delayed teenagers.

A70

Role of oncology/genetics nurse in management of individuals with hereditary diffuse gastric cancer
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2)A70

Background: Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant, rare familial cancer syndrome in which affected individuals develop diffuse gastric cancer at a young age. Mutations in the E-cadherin gene (CDH1) have a 70% lifetime risk of becoming gastric cancer and women with the gene have a 20-40% risk of developing lobular breast cancer.

Case study: This paper will report on a series of young adults who have undergone predictive genetic testing and been identified as carrying the CDH1 mutation known to be present in their family. The majority of these patients have been diagnosed with gastric cancer at their first screening gastroscopy.

Nursing intervention: Individuals who are known to carry the mutation are provided with a personalised cancer risk management plan with the aim of reducing morbidity overwhelmed by the number of appointments they receive and by the variability of information given to them, as care often moves from and between several teams. A care pathway has been developed by the oncology/genetics nurse to ensure optimal communication between the genetics and oncology treatment team, to ensure the best outcome for the patients. The oncology/genetics nurse coordinator with her specialist knowledge of genetics plays a vital role in facilitating ongoing follow up of these individuals, coordinating screening, educating patients and their family on risk management strategies.

Conclusion: This paper will provide a clinical update on this rare familial syndrome will describe the needs of patients and families affected by this condition and illustrate the contribution that expert nursing care makes to the outcomes of people affected by HDGC.

A71

The challenges of finding the gene responsible for a rare, autosomal dominant gastric cancer susceptibility syndrome
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2)A71

Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS) is a newly described, rare autosomal dominant syndrome, characterized by fundic gland polyposis with occasional hyperplastic and adenomatous polyps. We diagnosed GAPPS in a large Australian (Family 1) and two smaller North American families (Family 2 and 3). Mutations in APC, MUTYH, CDH1, SMAD4, BMPR1A, STK11 and PTEN were excluded in all families by sequence analysis of exons and flanking regions, as well as by assays for deletion or duplication of exons. We mapped the GAPPS gene in Family 1 by linkage analysis (LOD score 4.21) to a 20Mb region which contains about 60 genes. Short tandem repeat genotyping showed that the affected members of Family 2 share a haplotype in this 20Mb region, but analysis of Affymetrix SNP 6.0 data from three affected members of Family 2 showed that they also shared several other regions of the genome. However, both Family 2 and 3 are too small for definitive linkage analysis.

We have carried out full exome sequencing for three affected individuals in Family 1 (and targeted sequencing of the linked region in another individual), and of three affected members of Family 2. We did not find any rare coding or splice site variants in the linked region that were shared by all affected members of Family 1; nor any from the same region in both affected members of Family 2. All coding exons and miR genes in the linked region have been sequenced at least 30X at every base, or by Sanger sequencing, except for seven exons for which Sanger sequencing is yet to be completed. We will also use Sanger sequencing to improve the coverage of exons in the linked region in Family 2.

However, our current hypothesis is that non-coding mutations in the linked region are responsible for the GAPPS syndrome in both Families 1 and 2 and so whole genome sequence analysis for two affected members in Family 1 has been performed and analysis is underway.
A72

The InSiGHT approach to classification of mismatch repair gene variants
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A72

The International Society for Gastrointestinal Hereditary Tumours (InSiGHT) is committed to the sharing of MMR variant information through the publicly accessible InSiGHT database. This amalgamation of various types of data related to MMR variants and Lynch Syndrome encompasses family history, tumour pathology, genotype, RNA, in silico and in vitro information sourced from published literature and submissions from various centers. InSiGHT is collaborating with organisations including those with national MMR data-sets, in order to centralise and make public the full extent of Lynch Syndrome associated variants. It is also InSiGHT’s goal to increase the number of submissions from individual labs, and encourages scientists from around the world to participate in this effort.

To understand the clinical impact of these variants, InSiGHT is bringing together an international group of experts to develop qualitative classification rules and apply them to variants of uncertain significance. The application of these classification rules to variants is a work in progress, with input from specialists in a diverse range of fields, and will enable thorough analysis of the available data. This process will culminate in regular teleconferences of a panel of experts who will discuss each variant and reach consensus on the clinical significance. Subsequent publication of the outcome will be of particular relevance to the medical genetics community.

A73

An incidental finding of a large genomic deletion of BRCA1 on a molecular karyotype for a 5 year old child
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A73

Background: The use of microarray based molecular karyotyping for diagnostic testing is now common in clinical practice. A feature of this technology is an increased capacity to uncover genetic abnormalities unrelated to the original indication for the test. These incidental findings can involve the unexpected diagnosis of well recognised Mendelian genetic disorders through the rearrangement of a known disease genes. Large scale genomic rearrangements of BRCA1 make a significant contribution to the molecular pathology of familial breast and ovarian cancer and are potentially detectable by the CGH or SNP microarrays in common use.

Case report: We describe the case of a 5 year old boy seen at the Peter MacCallum Familial Cancer Centre. The index case and his twin brother originally presented to their pediatrician for investigation of a history of large scale genomic rearrangements of BRCA1. This array revealed an unexpected finding of a deletion on chromosome 17q21 involving the BRCA1 gene locus. Analysis showed that the copy number variant resulted in the deletion of exons 2-17 and would be classified as a pathogenic mutation. Following discussion with a clinical geneticist the result was reported to the family by the pediatrician who facilitated their referral them to a familial cancer centre for further discussion.

A detailed pedigree revealed no significant cancer history with only a single case of breast cancer in the wider family. The parents of the index described being surprised by the result but grateful for the information. Both parents elected to proceed with predictive testing to clarify the origin of the mutation and the potential cancer risks for them and their family in the future.

Conclusion: The rapid expansion of genetic testing capacity through new technologies such as microarray testing or next-Generation-Sequencing are certain to be associated with increasing numbers of unexpected findings including familial cancer syndromes. Although an approach to this type of finding needs to be developed, the current case demonstrates that whilst unanticipated, this information can be welcomed by families.

A74

Metachronous colon cancer risk following surgery for first primary rectal cancer in Lynch syndrome
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A74

Background: It is known that metachronous colorectal cancer risk for Lynch syndrome patients with primary colon cancer is high and total colectomy is the preferred option [1]. However if the index primary cancer is in the rectum, management advice is complicated by considerations of worsening bowel function or stoma formation. To aid surgical decision-making, we estimated the risk of metachronous colon cancer for Lynch syndrome patients who underwent either anterior resection or abdominoperineal resection for primary rectal cancer.

Methods: This retrospective cohort study comprised 79 MMR gene mutation carriers (18 MLH1, 55 MSH2, 4 MSH6 and 2 PM2) from the Colon Cancer Family Registry who had a surgical resection for their first primary rectal cancer. Age-dependent cumulative risks of metachronous colon cancer were calculated using the Kaplan-Meier method. Risk factors for metachronous colon cancer were assessed using a Cox proportional hazards regression.

Results: During 866 person-years of observation (median 9 years; range 1-32 years) since diagnosis of first rectal cancer, a total of 21 (27%) carriers were diagnosed with metachronous colon cancer (incidence 24.2; 95% CI 15.8–37.2 per 1000 person-years). Incidence for carriers who had an anterior resection (26.8; 95% CI 15.5–46.1 per 1000 person-years) was not different from that for carriers who had an abdominoperineal resection (21.0; 95% CI 10.5–42.1 per 1000 person-years) (P=0.1). Cumulative risk of metachronous colon cancer was 19% (95% CI 9.3–31%) at 10 years, 47% (95% CI 31.6–68.1%) at 20 years and 69% (95% CI 45–89%) at 30 years after surgical resection. There was no difference in the frequency of surveillance colonoscopy between two types of surgery (one colonoscopy per 1.1 (95% CI 0.9–1.2) years after anterior resection vs. one colonoscopy per 1.4 (95% CI 1.0–1.8) years after abdominoperineal resection).

Conclusions: For carriers of MMR gene mutations who contract rectal cancer, the metachronous cancer risk is substantial and mirrors that seen for carriers undergoing segmental resection for primary colon cancer [1], despite the majority continuing to receive frequent surveillance colonoscopy. This risk needs to be considered when the extent of surgery for primary rectal cancer is planned. Whereas total colectomy for primary colon cancer in mutation carriers is appropriate, for primary rectal cases this strategy has major implications for continence and need for stoma. Nevertheless, given the high metachronous risk, this needs serious consideration especially for younger patients.

Reference
Colorectal tumour BRAF V600E and MLH1 promoter methylation status in the assessment of mismatch repair gene sequence variants of unknown clinical significance

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Lynch Syndrome is the most common hereditary cause of colorectal cancer, caused by pathogenic mutations in the mismatch repair (MMR) genes that result in functional defects in the DNA mismatch repair complex. Individuals identified with a pathogenic mutation are at high-risk of early onset colorectal and/or endometrial tumours. However, up to 50% of MMR sequence variants are reported to be of unknown clinical significance, creating a problem for clinicians and patients. We are developing a multifactorial model to assess the pathogenicity of MMR gene sequence variants of unknown clinical significance. Currently this model utilizes data on tumour microsatellite instability (MSI) and family segregation analysis to assess the likelihood that a variant carrier demonstrates features expected for a carrier of a pathogenic mutation. Tumour BRAF V600E mutation and MLH1 promoter methylation are reported to be associated with MSI-H, sporadic colorectal cancer and may thus provide a strong prediction that a tumour with MSI-H status is not from a MMR gene mutation carrier.

A literature review of BRAF V600E and MLH1 promoter methylation in colorectal cancer patients was performed. Frequency of these tumour features in each study was assessed, stratified by MSI status and reported/likely MMR gene mutation status. Frequency of these characteristics was also analysed in the Colon CFR dataset, and used to estimate a likelihood ratio (LR) of pathogenicity for use in multifactorial modelling.

As expected, the literature review of 4655 individuals from 33 studies revealed a high frequency of BRAF mutations in MSI-H tumours without a MMR mutation, which increased for the subset of cases with lack of MLH1 tumour protein expression. However, BRAF V600E mutation was identified in one patient with a PMS2 mutation, challenging previous assumptions that BRAF mutation status excludes positive MMR gene mutation status. MLH1 promoter methylation data generated using a variety of experimental designs were available for only 2984 individuals from 40 studies, with inconsistencies in frequency between studies. Information was reported on methylation status of 204 known MLH1 gene mutation carriers. Analysis of the Colon CFR dataset showed that BRAF tumour mutation status predicted MMR gene mutation status when combined with tumour MSI-H status. However, MSI-L and MSS with BRAF did not provide any additional predictive power over MSI-L or MSS status alone. The likelihood of a tumour with MSI-H and BRAF V600E positive status to be from a non-carrier was 11.55 and 9.85 for population-based and clinic-based patients, respectively. However, 4/98 (4.08%) of MSI-H BRAF positive tumours were from MMR mutation carriers. In conclusion, positive BRAF tumour status is not a conclusive test to exclude MMR gene mutation status for patients, but BRAF-MSI tumour status is a powerful addition to multifactorial models evaluating clinical significance of MMR gene variants.

Loss of MSH6 and PMS2 immunohistochemical staining in tumour tissue of two individuals with a germline PMS2 mutation

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Lynch syndrome is an autosomal dominant cancer predisposition syndrome which is caused by a germline mutation in one of four genes, MLH1, MSH2, MSH6 or PMS2. Individuals with a germline mutation in one of these genes are at increased lifetime risk of colon, endometrial, ovarian, small intestine, renal pelvis and ureter. Less commonly patients may develop biliary tract cancers, gastric and pancreatic cancers, brain tumours, sebaceous adenomas, carcinomas and skin keratoacanthomas. Immunohistochemical staining for the above four mismatch repair (MMR) proteins is routinely performed for individuals with bowel cancer or a related cancer who are suspected of having Lynch syndrome. This helps target genetic testing to the correct mismatch repair gene. The genes involved in Lynch syndrome work together in a DNA repair complex. The MMR proteins form a heterodimer with MLH1 partnering PMS1, PMS2 or MLH3 and MSH2 partnering MSH3 or MSH6. This means that a mutation in MLH1 leads to loss of staining of both MLH1 and PMS2, while a mutation in MSH2 leads to loss of staining of both MSH2 and MSH6. The same is not true however, for mutations in either PMS2 or MSH6, where only the single protein made by these genes is not expressed in the tumour.

However, the pattern of staining using immunohistochemistry is sometimes more complex due to coding microsatellites within the MSH6 gene. We present two unrelated cases with a germline PMS2 mutation which had loss of both MSH6 and PMS2, but normal MLH1 and MSH2 on immunohistochemical staining.

State-wide population screening for Lynch syndrome? Improved ascertainment of at risk families

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We have previously established in a large retrospective study that MSI testing was an effective first screen for the identification of individuals with Lynch syndrome (LS) in colorectal cancer (CRC) patients aged < 60 years. From these findings, we and/or IHC screening was recommended for all newly diagnosed CRC patients aged < 60 years in Western Australia, regardless of family history of cancer. We have subsequently evaluated the utility of routine MSI/IHC screening in diagnostic pathology laboratories for the detection of previously undiagnosed individuals and families with LS. From January 2009 to December 2010, 270 tumours were tested for MSI and expression of MLH1, PMS2, MSH2 and MSH6 using IHC. Cases showing MSI and/or loss of expression were also tested for BRAF V600E mutation. Seventy cases were found to have MSI, of which 25 were excluded from further investigation as possible LS cases due to the presence of the BRAF V600E mutation. The remaining 45 “red flag” cases were eligible for germline testing based on their MSI, IHC and BRAF status. From 26 cases tested to date, 11 germline mutations have been found. Nine were from individuals not previously recognized as LS and two were untested members from known LS families. Extrapolation of the mutation incidence (11/26, 42%) to all red flag cases (n=45) suggests approximately 19 mutation carriers in this cohort. This value approximates the number of LS cases that could be expected to arise in the Western Australian population over a two-year period (n=24), assuming that 1% of all CRCs are due to LS. Our preliminary findings following the implementation of state-wide routine MSI and IHC testing in Western Australia indicate that the majority of LS cases are being identified.

Audit of routine immunohistochemistry testing for mismatch repair proteins at diagnosis of colorectal cancer under the age of 50

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Background: In May 2007, the Victorian Cancer Oncology Hereditary Bowel Cancer Group (VCOG HBCG) released a position statement in regards to the identification of Hereditary Non-Polyposis Colorectal Cancer (HNPPC) by immunohistochemistry (IHC) testing. This was based on the consensus among clinical groups, that most families with HNPPC are not being identified and strategies to improve identification should be implemented. The VCOG HBCG recommendations was to test all colorectal cancers in patients under 50 years of age by IHC for MLH1, MSH2, MSH6 and PMS2 proteins, as part of the routine pathological assessment of cancers presenting in these patients, without direct consent. This recommendation was supported by the Australian College of Pathologists and widely circulated to the clinical community from 2007.

The primary purpose of this audit was to ascertain the frequency of IHC being performed for consecutive patients diagnosed with colorectal cancer under 50 years of age, at three Victorian hospitals since the publication of the VCOG HBCG position statement. The purpose of this audit was also to ascertain the number of cases where IHC results showed no evidence of expression, which were referred to the Familial Cancer Centre (FCC) for further assessment and the outcome of this assessment.

Methods: Lists of patients with colorectal cancer diagnosed under 50 years of age for the calendar years of 2007, 2008, 2009 and 2010 were extracted from each hospital database. Pathology reports for all patients were manually checked to assess cancer site, stage, mucinous component, tumour infiltrating lymphocytes and whether IHC was performed. Those patients with IHC absent results were cross-checked with FCC databases to see whether they were referred for further evaluation and what the outcome of attendance had been.

Results: Data from three hospitals is presented. 204 patients were diagnosed with CRC under 50 years of age from Jan 2007 to Aug 2010. 164 had IHC testing as part of routine management: 67.5% (27/40) in 2007, 69.8% (44/63) in 2008, 66.6% (36/54) in 2009 and 72% (36/50) in 2010. One hospital showed a greater increment in rate of IHC uptake over time. This hospital changed its synoptic pathology report to include IHC.

Conclusion: There were differences between hospitals in the uptake of the recommendation to implement routine IHC for patients diagnosed with colorectal cancer under 50 years of age. Barriers and enablers were identified for initiating routine IHC testing at the hospitals. These include addressing concerns about consent, and the cost of consent acquisition if needed, confusion about whether the IHC testing is a genotype or phenotype test, translation of the findings to clinical practice, establishment and optimization of the IHC testing, and funding of IHC testing.

A79 Bone density loss after risk reducing salpingo-oophorectomy

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Women undergoing risk-reducing salpingo-oophorectomy through the Prince of Wales Hospital Hereditary Cancer Clinic are offered enrolment in a prospective study of bone mineral density (BMD), markers of bone metabolism and cardio-vascular risk factors. Participants are invited to enroll a friend who has ovaries in situ and are aged within five years of the participant as a control population.

Lumbar spine (LS), femoral neck (FN) and total body (TB) bone mineral density changes as a percentage annual loss are reported and compared with age matched norm. Women who were pre-menopausal at the time of oophorectomy have been compared with those who were either already post-menopausal at oophorectomy or had ovaries in situ to evaluate the impact of this intervention.

59 women have been enrolled and have now had 2 or more bone density evaluations – 44 cases and 15 controls. 7 women who were taking HRT or drugs affecting bone metabolism at entry were excluded from this analysis. The average age of women who had undergone pre-menopausal RRSO was 48.4yrs and 49.4yrs for the comparison group. Of those who were still menstruating at the time of RRSO and were not taking HRT at enrolment, the average annual decrease in LS BMD from year 1 to year 2 was 1.3% of enrolment value, in FN was 0.95% and in TB BMD was 0.6%. The corresponding decrease for the comparison group was 0.8% in LS, 1.3% in FN and an increase of 0.26% for TB. Both groups are aged under 50 and demonstrate a greater than average BMD loss compared with age matched norms (population average annual LS loss is 0.35% in pre-menopausal women and 0.9% for women in early menopause [1]).

The small cohorts so far do not demonstrate any statistically significant differences, but recruitment is ongoing. These findings are consistent with established data which has shown that LS has the greatest loss of BMD in the early menopausal years, with stable or even increasing whole total body BMD.

Data will be presented on total body fat and fat distribution changes in these cohorts.

Reference

A80 Primary treatment patterns in women recruited to the Australian Ovarian Cancer Study

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Ovarian Cancer Study

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http://www.hccpjournal.com/supplements/10/S2

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In conclusion, the majority of women in AOCs were treated according to national guidelines, enabling the selection of cases with uniform treatment for projects investigating associations of genomic and genetic features with clinical outcome.

**Reference**


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**A81**

**Integrated genomic analysis and functional characterisation of novel oncogenes in ovarian cancer**

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**Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A81**

Ovarian cancer is associated with the highest mortality rate of all gynaecologic malignancies, and is identified as the sixth most common cause of cancer death for Australian women. Genomic copy number amplification is a hallmark of oncogene associated tumour development and progression. The targeted increase in copy number of such chromosomal regions has a significant impact upon gene expression that imparts selective advantages on cancer cells. These over-expressed genes are attractive therapeutic targets, particularly as increased gene expression within the cancer genome is often associated with ‘oncogene addiction’.

To identify amplonic targets we have adopted a methodology that combines genomic copy number and expression data with RNA interference (RNAi) to identify amplified genes that are functionally relevant. According to our analysis of SNP array data over 46 Mb of the genome was amplified as a high level in 10% or more tumours, encompassing ~300 genes. This set of candidate genes will be functionally assessed in vitro using a boutique siRNA library to interrogate phenotypic alterations in proliferation and morphology. We have utilized SNP6.0 array data from 39 ovarian cell lines to identify 18 cell lines that recapitulate the gene amplifications observed in clinical specimens, which form the basis of this study.

Data obtained from the first cell lines examined indicates that despite the different genetic backgrounds of the cell lines used, there is overlap in the genes that cause significant alterations to cellular proliferation. Furthermore, a number of genes not previously associated with ovarian cancer have been shown to impart functional effects directly associated with genomic amplification. This data provides evidence that the high-throughput nature of this functional screen will rapidly identify candidate genes that may then be streamlined to a validation phase. The identification and validation of such genes is critical to their translation as potential therapeutic targets.

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**A82**

**Pathological diagnosis of ovarian cancer**

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**Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A82**

Case 1: Serous tubal cancer detected on PAP smear: A 60 year old woman with a history of breast cancer was referred after malignant cells were found on PAP smear. Abdominal exam, cervical visualisation, transvaginal ultrasound and CT of the abdomen and pelvis were all normal. Endocervical and endometrial curettings demonstrated serous carcinoma cells consistent with tubal / ovarian origin. CA125 was slightly elevated at 53 (Upper Limit of Normal 35).

At laparotomy uterus, fallopian tubes and ovaries appeared normal. She had a hysterectomy, bilateral salpingo-oophorectomy, omentectomy, peritoneal biopsies and washings. There was no macroscopic evidence of cancer, but microscopic examination confirmed serous cancer in the fimbrial end of the left fallopian tube(A) with scattered malignant cells in the lumen of both fallopian tubes(B), the omentum(C) and surface of the ovaries(D), surface of uterus and paracolic gutter. Washings were positive. Family history was unremarkable. She underwent BRCA1 mutation screening which revealed a BRCA1 mutation. Despite intraperitoneal cisplatin and paclitaxel she relapsed and died 4 years later.

**Case 2: Positive washing after RRSO in a BRCA 1 mutation carrier:** A 40 year old woman had been diagnosed and treated for breast cancer at age 18. Mutation testing revealed a BRCA 1 mutation. She elected to have risk reducing salpingo-oophorectomy and hysterectomy at age 40. The fibria, fallopian tubes and ovaries were examined at 3mm intervals and immunohistochostmetry was performed. The right fallopian tube was found to contain serous tubal intraepithelial carcinoma (STIC) with diffuse p53 staining (E) and Ki-67 seen in 50% of lesional nuclei (F). Washing were positive for adenocarcinoma and were similar in appearance to the serous tubal intraepithelial carcinoma. The left fallopian tube was found to have a precursor lesion with a p53 signature.

After discussion with her treating oncologist, and review of the case within the multidisciplinary tumour board, she elected to undergo adjuvant chemotherapy for ovarian cancer.

**Case 3: Right adnexal mass in residual right fallopian tube following bilateral oophorectomy:** A 60 year woman presented with a right sided pelvic mass. In 2001 at age 48, she had been diagnosed with breast cancer. She underwent BRCA 1 or 2 Jewish founder mutation testing which revealed a BRCA1 mutation. She subsequently had risk reducing bilateral oophorectomy.

At laparotomy, the pelvic mass was a right adnexal mass which was a poorly differentiated serous carcinoma involving the right fallopian tube.

**Discussion:** Knowledge of origin and evolution of ovarian cancer in BRCA mutation carriers has lead to changes in protocols of risk reducing surgery is performed and how specimens from risk reducing surgery are handled. A more intensive sectioning and staining as well as examination of peritoneal washing is now standard in most labs. These cases illustrate the tubal origin of BRCA-related serous cancers and the propensity for very early dissemination. In addition, pathology from previous gynaecological surgery should be reviewed to ensure complete removal of ovaries and fallopian tubes.

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**A83**

**Benign serous ovarian tumour: a redefining moment?**

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**Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A83**

While the paradigm that malignancies arise through a stepwise progression from benign precursors has been established for many malignancies, it remains unclear if this holds true for ovarian cancer. Serous ovarian carcinomas are the predominant clinically important subtype and it has been widely believed that some or all of these arise from precursors derived from the ovarian surface epithelium, such as inclusion cysts or serous benign and borderline tumours. Despite the co-occurrence of benign, borderline and low grade carcinoma epithelial components, direct molecular evidence for the benign lesions as precursors is limited. This study aimed to perform high resolution copy number analysis using a series of benign serous ovarian tumours to identify any underlying genomic changes indicative of early events in tumourigenesis, which could assist in determining if these lesions represent precursors to some invasive serous ovarian carcinomas. This is the first ultra-high resolution copy number analysis of benign serous tumours of the ovary.
High resolution copy number analysis was performed on tumour epithelial and fibroblast DNA using the Affymetrix OncoScan and SNP 6.0 array platforms. Copy number aberrations (CNAs) were detected in the epithelium of only 5.6% (2/35) of serous cystadenomas and cystadenofibromas. Unexpectedly, CNAs were detected in the tumour fibroblasts in 36% (14/39) of cases, including gain of chromosome 12 in 10 cases. No KRAS or BRAF mutations were detectable in either component of the benign serous tumours. Chromosome 12 trisomy has been previously identified in pure fibromas, supporting the concept that a significant proportion of benign serous tumours are in fact primary fibromas with an associated cystic mass. This study therefore provides a novel perspective on the development of these tumours.

A84
Analysis of RAD51C germline mutations in high-risk breast and ovarian cancer families and ovarian cancer patients
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A84

The recent identification of a biallelic RAD51C (FANCO) mutation in a family with a Fanconi Anemia-like disorder led to its examination in a large hereditary breast and ovarian cancer case-control candidate study (Meindl et al., 2010; Vaz et al., 2010). Meindl et al. identified six independent pathogenic monoallelic mutations. Interestingly, these mutations were identified exclusively within 480 families with breast and ovarian cancer (frequency 1.3%) but not among any of the 620 families with breast cancer only. In most subsequent studies, the mutation frequency has been found to be lower than 1.3%, with only three additional truncating mutations being identified in five families among more than 729 ovarian cancer families (with or without breast cancer) (Akbari et al., 2010; Pang et al., 2011; Romero et al., 2011; Silvestri et al., 2011; Wong et al., 2011; Zheng et al., 2010). Despite the presence of breast cancer in 10 of the 15 RAD51C mutation positive families reported to date, analysis of more than 1,373 breast cancer-only families by eight studies collectively has not identified any additional families with truncating RAD51C mutations. Therefore, while evidence of a causative role for RAD51C in breast and ovarian or ovarian only cancer families (HBOC) is convincing, albeit with low prevalence, its role in breast cancer only (HBC) families remains unclear.

To provide more definitive data on the incidence of RAD51C mutations in hereditary breast and ovarian families, we utilised high resolution melt (HRM) analysis to screen for germline mutations in all coding exons of RAD51C in index cases from 1,388 non-BRCA1, non-BRCA2 high risk Australian HBC and HBOC families, and 427 controls. In addition, the contribution of RAD51C in unselected ovarian cancer was examined through the analysis of germline DNA from an unselected cohort of 267 ovarian cancer patients.

Analysis of 1,053 HBC and 335 HBOC families, and 267 unselected ovarian cancer cases identified 12 novel heterozygous variants in RAD51C, three of which were protein truncating, six non-synonymous, one synonymous and two non-coding. Numerous dbSNPs and variants from previous studies were also identified. Two truncating mutations, c.72-73insTGGCGG (p.V25fsX3) and c.397C>T (p.Q133X), were identified amongst the 1,388 familial cases. Consistent with the previously reported deleterious mutations, both variants were identified in families with at least one report of ovarian cancer. A third truncating variant, c.230delG (p. V77fsX24), was identified in a high grade serous tumour among the 267 unselected ovarian cancer cases. In silico analyses predict that four missense variants (including two novel variants) are likely to be pathogenic. Our data also provide support for the designation of the previously reported missense variants p.G264S, and possibly p.A126T, as moderate penetrance alleles.

A85
The use of the Illumina FFPE Restoration Protocol to obtain suitable DNA for SNP-based CGH – a pilot study
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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A85

The use of formalin fixed paraffin embedded (FFPE) tissues for DNA analysis has been met with great difficulties due to the degradative effect this fixation, processing and storage has on DNA quality. The lack of a suitable protocol to enhance the quality of such degraded DNA has been a great hindrance to our ability to make full use of clinically annotated FFPE cancer tissue banks. In this report we have begun to investigate the effectiveness and limitations to Illumina’s recent platform for the restoration of DNA derived from archival FFPE tissues.

With the exception of select fresh frozen and blood samples, all DNA samples were extracted from FFPE tissues and restored according to Illumina’s protocol. The quality of the FFPE extracted DNA was then assessed by Illumina’s PCR-based quality control assay (QC-PCR) and the resultant DNA was subsequently used in Illumina’s SNP-based CGH chips. Chip call rates were largely used in order to determine the quality of a particular array. Overall, the chip data were highly reproducible as determined by comparing several technical replicate samples. FFPE-extracted and restored DNA performed well in comparison to DNA extracted from fresh frozen tissue and blood from the same patients, meeting the minimum standard for continuation of this platform. It should also be noted that the quality of the chip data was not appreciably enhanced by the use of sodium thiocyanate during the extraction of DNA from FFPE tissues. Moreover, chip quality was significantly lower, with regards to call rates and ‘quality’ of b allele frequency plots, when the recommended Roche DNA extraction kit was used instead of the Qiagen DNA extraction kit. Of great importance, we found that the QC-PCR provided an accurate prediction of chip quality as determined by comparing chip call rates with PCR signals derived from proprietary primer sets (rho=0.6242, p<0.0001). The selection of restored DNA for future studies will be guided by the results from the QC-PCR assay.

These preliminary data demonstrate the promise of Illumina’s DNA restoration protocol for FFPE extracted DNA from tissues older than three decades. Further studies are required to determine the full potential of this method for SNP-based CGH analysis of large FFPE tumor banks.

A86
Functional polymorphisms in the TERT promoter are associated with risk of serious ovarian and breast cancer
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A multi-centre international quality control study comparing mRNA splicing assay protocols and reporting practices from the ENIGMA consortium

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A87

Classification of intronic and predicted missense changes in the breast cancer susceptibility genes BRCA1 and BRCA2 remains a signiﬁcant challenge for management of patients carrying these variants. Defective mRNA splicing is established as a pathway to disease, and mRNA analysis of unclassiﬁed variants has been shown to assist in classiﬁcation and genetic counselling. However the interpretation of splicing assay results can be diﬃcult, particularly for those variants that give rise to aberrations in a background of naturally occurring isoforms.

The ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) consortium was set up to facilitate research and improve research methods used to classify rare variants in the BRCA1 and BRCA2 (and potentially other) breast cancer predisposition genes. ENIGMA has established a Splicing Working Group, with the purpose to pool the expertise of diﬀerent active research groups to conduct large-scale studies that improve the clinical classiﬁcation of likely spliceogenic variants. An initial project of the Splicing Working Group is to assess the consistency of protocols and results obtained across the multiple participating laboratories from Australia, Europe, UK and the USA.

A comparison of mRNA assay protocols in use across 21 labs has identiﬁed diﬀerences in source material for RNA assays (cultured and uncultured lymphocytes, lymphoblastoid cell lines (LCLs) or constructs), diﬀerential use of nonsense-mediated decay inhibitors, and numerous differences in mRNA extraction, DNase treatment and cDNA synthesis methods. A second phase of the project is now underway to determine the impact of the splicing assay methods routinely used by these laboratories on assay data and clinical interpretation of a panel of variants. LCLs were selected from the kConFab repository from carriers of a variant associated with single major aberrant mRNA transcript absent in controls (n=4); carriers of a variant associated with a complicated aberrant mRNA splicing proﬁle involving multiple transcripts including naturally occurring isoforms (n=5); female cancer-free controls (n=11).

LCLs have already been distributed to 15 of 20 participating sites, and mRNA assays are underway. Preliminary results indicate that major aberrations associated with several variants mirror results previously observed for mRNA from uncultured lymphocytes. In addition, there is evidence for notable diﬀerences in expression of some isoforms compared to results previously observed for RNA from uncultured lymphocytes. This collaborative eﬀort will provide information to inform optimal standardised mRNA splicing assay methodology, and to improve guidelines for clinical interpretation of assay results.

Identification of a novel disease-associated variant in the BRCA1 3’UTR that introduces a functional miR-103 target site

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A88

Mutations in the breast cancer susceptibility genes, BRCA1 and BRCA2, represent the majority of the known familial breast cancer risk, yet
account for only 20% of the total risk. As BRCA1 is a large gene, genetic screening of high-risk individuals is limited to the coding regions and intron-exon boundaries, which precludes the identification of mutations in non-coding and untranslated (UTR) regions. Although mutations within 3′UTRs have been identified in many genes and are known to influence cancer susceptibility through the disruption or creation of protein and microRNA binding regions, mutation analysis of the BRCA1 3′UTR to date has been very limited. In this study, we screened the BRCA1 3′UTR for potential regulatory mutations. Using a large cohort of 1,585 BRCA-mutation negative, breast cancer cases, we identified seventeen novel BRCA1 3′UTR variants, eight of which were identified in breast cancer cases and absent in a large panel of cancer-free controls. Four of these variants, c.*S8C>T, c.*S28G>C, c.*718A>G, and c.*1271T>C, significantly reduced 3′UTR associated regulatory activity, as measured by reporter assays using MDA-MB-231 breast cancer cells. In addition, three BRCA1 3′UTR variants, c.*718A>G, c.*800T>C and c.*1340_4220 Tdel, were predicted to create new miRNA binding sites. Of these, c.*1340_4220 Tdel showed a significant reduction (25%, p=0.0007) in luciferase activity when co-expressed with the predicted targeting miRNA, mir-103 in MCF-7 cells. This is the most comprehensive set of BRCA1 3′UTR variants published to date and highlights the importance of cataloguing 3′UTR variants for functional analyses and cancer risk association.

A89

The contribution of LARGE genomic rearrangements of BRCA1 and BRCA2 gene mutations in breast and ovarian cancer families in a clinical cohort

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A89

Background: The use of multiplex ligation-dependent probe amplification (MLPA) to detect large scale rearrangements is now a standard component of BRCA1 and BRCA2 gene testing in the clinical setting. With the cost of full Sanger sequencing up to 4 times higher than the cost of MLPA, it is important not only to determine the prevalence of these mutations but to ascertain the probability that a family may harbour a large deletion or rearrangement in the BRCA1 and BRCA2 genes. Here we examine the incidence and clinical associations of genomic rearrangements in the BRCA1 and BRCA2 genes in a cohort of index cases from high risk breast and ovarian cancer families recruited from familial cancer centres (FCC).

Method and results: The Victorian FCC Translational breast cancer cohort includes 1222 index cases identified from families who had been seen through one of the four FCCs in Victoria. Until 2007, standard BRCA tests did not include MLPA but instead used a variety of sequencing-based methods which included FTT, DHPLC and Sanger sequencing. Of these cases, 246 (20.1%) were found to carry a BRCA1 or BRCA2 mutation using sequencing-based methods. In a small proportion of cases MLPA was performed prior to study commencement based on clinical indications leading to the detection of 11 mutations. A total of 965 cases were found not to carry a BRCA1 or BRCA2 mutation through sequencing-based methods and were eligible for MLPA in the study. A hundred and nine cases were excluded from testing: 19 did not fit inclusion criteria, 93 had unusable DNA for testing.

In the remaining 856 cases a further 24 (2.8% of cases) BRCA1 and BRCA2 mutations were identified using MLPA. In the total cohort of 1113 index cases, 246 (22.1%) BRCA1 and BRCA2 mutations have been identified, including 36 (14.2% of mutations) large deletions and duplications detected through MLPA. Mean age of onset for first breast cancer diagnosis was 41 years (26-73) in mutation carriers detected through sequencing based methods and 40 years (18-60) in cases with genomic rearrangements detected by MLPA. Analysis of the BRCAPRO scores revealed that the mean BRCA carrier probabilities for BRCA mutation carriers detected though MLPA were significantly higher than those detected using sequencing based methods (0.58 versus 0.37 respectively, p=0.002). Further analysis of correlations between mutation type and patient demographics including the cancer profile of the index case and their 1⁵⁻³⁸ degree family members, rates of bilateral breast cancer, male breast cancer and early age of onset will be presented.

Conclusion: MLPA detected genomic rearrangements accounted for 14% of all BRCA mutations in a large cohort of Victorian FCC families. The association with higher pre-test carrier probabilities indicates that an optimal strategy for BRCA mutation detection in which an initial MLPA screen in high risk families may avoid the need for sequencing in some patients where a genomic rearrangement is present, with an associated cost saving.

A90

Contribution of genetic variation within SuprMam1 and SuprMam2 to breast cancer susceptibility

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Hereditary Cancer in Clinical Practice 2012, 10(Suppl 2):A90

Two recessive mammary tumour susceptibility loci, SuprMam1 (for suppressor of mammmary tumours) on chromosome 7 (110-140 Mb) and SuprMam2 on chromosome 2 (120-140MB) have been identified in the BALB/c mouse strain in the Trp53+/- mouse model of spontaneous breast cancer [1]. We studied mammary gland morphology and expression levels of potential candidate genes in SM09 congenic mice (BALB/c SuprMam loci in C57BL/6 background) in comparison to parental strains, to identify the genes within the SuprMam loci that might be responsible for higher cancer susceptibility in BALB/c mice.

We analysed mammary gland wholemounts for differences in their basic morphology. The average of ductal branch count per 4x field of view was, BALB/c (diestrus) =171, BALB/c (estrus) =173, C57BL/6 (diestrus) =43, C57BL/6 (estrus) =38, SM09 (diestrus) =29 and SM09 (estrus) =23. According to these results, even though there is a significant difference between the numbers of ductal branches between the two parental strains BALB/c and C57BL/6 (estrus:p=0.002, diestrus:p=0.027), there is no significant difference between congenic (SM09) and control (C57BL/6) mice during either estrus (p=0.164) or diestrus (p=0.299) stages at 12 months of age. A similar pattern was observed for total epithelial area. This suggests that BALB/c SuprMam alleles alone do not contribute significantly to the morphological differences of the parental strains.

The tumour suppressor DMBT1 (deleted in malignant brain tumors) has previously been identified as a candidate modifier gene within SuprMam1 (1). Using semi-quantitative RT-PCR, Dmbt1 mRNA was found to be significantly lower in mammary glands of susceptible BALB/c mice when compared to C57BL/6, while SM09 congenic mice had similar levels to the control C57BL/6 mice. This indicates that the lower level of Dmbt1 expression in BALB/c mice must be due to transcriptional factor differences outside the region, which is not present in SM09 congenics.

Using Affymetrix data comparing T cell gene expression across 5 different mouse strains, Cyp2r1, a major vitamin D hydroxylase, was identified as another potential candidate gene in SuprMam1. Using semi-quantitative RT-PCR, Cyp2r1 mRNA was found to be 9.44 nM/L, SM09= 59.44 nM/L. Surprisingly, however, this is the opposite of what is expected based on the association of CYP2R1 expression and cancer susceptibility in human populations.

With these results, we are able to rule out two important candidate genes, DMBT1 and CYP2R1, from being responsible for the higher susceptibility of SuprMam1. In order to confirm these results, and also to identify the possible involvement of other selected candidate genes, we aim to carry out transcriptional profiling on mammary glands of SM09 congenic and control mice.
Early exploration of two candidate breast cancer susceptibility genes identified by whole-exome sequencing

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A91

Breast cancer (BC) is the most frequently diagnosed cancer in women around the world, and an estimated 15–20% of BC cases present with a family history of disease. Genetic variants in known susceptibility genes explain a relatively small proportion of the heritable risk for BC. Genetic variants have been broadly classified into three categories with different levels of risk and prevalence: rare mutations in high-risk genes (e.g., BRCA1, BRCA2); rare mutations in intermediate-risk genes (e.g., CHEK2); and common very modest-risk genetic variants (identified throughout the genome). These categories currently account for 20%, 5% and ~15% of the familial risk, respectively, leaving about 60% of the familial BC risk to be determined.

We have conducted whole-exome capture followed by massive parallel sequencing (XC-MPS)-based analysis on greater than third degree affected relatives from highly selected multiple-case BC families (at least four cases of invasive breast cancer diagnosed before 50 years) which had previously been screened and found not to carry identifiable BRCA1 or BRCA2 mutations. In this presentation, we focus on two candidate BC susceptibility genes identified by this analysis, each playing a key-role in DNA repair. In both cases, one individual from the pair of cousins was found to carry a predicted protein damaging genetic variant at a consensus splice site. The variants were confirmed and extended analysis within the respective pedigrees performed by Sanger sequencing, subject to DNA sample availability. Further characterization of these variants is being pursued by much larger scale case-control genotyping using the TaqMan platform.

Our work further illustrates the complexities of human genetic variation and the technical and analytical challenges of identifying variation that is associated with inherited predisposition to complex diseases such as breast cancer.

A92

Expanded genetic analysis of a PALB2 c.3113G>A mutation carrying multiple-case breast cancer family via exome sequencing

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A93

Identification of copy number alterations associated with the progression of DCIS to invasive ductal carcinoma

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A94

Ductal carcinoma in situ (DCIS) is a non-obligate precursor to invasive ductal carcinoma (IDC). Identification of the genetic differences between the two lesions may assist in identifying genes that promote the invasive phenotype. To annotate these alterations we analysed 21 breast tumours containing synchronous areas of DCIS and IDC. Tumour cells were microdissected from FFPE tissue and analysed by 300K Molecular Inversion Probe (MIP) copy number arrays. Matched IDC and DCIS showed highly similar copy number profiles (average of 83% of the genome shared). Four regions of loss (3q, 6q, 8p and 11q) and four regions of gain (5q, 16p, 19q and 20) were recurrently affected in IDC but not in the matching DCIS. CCND1 and MYC showed increased amplitude of gain in IDC. One region of loss (17p11.2) was specific to DCIS. Our data shows that DCIS is an advanced pre-invasive tumour with genetic instability and continues to evolve in parallel with co-existing IDC. In the IDC-specific regions of genomic alteration we have identified novel loci as well as genes with previous links to breast cancer progression.
Background: AOCS commenced in 2000 as a collaborative study between researchers at the Peter MacCallum Cancer Centre (PMCC), Queensland Institute for Medical Research (QIMR), Westmead Institute for Cancer Research (WICR) and University of Melbourne. Patient recruitment ceased on June 30, 2006. AOCS recruited a total of 1834 women with invasive or borderline ovarian cancer, far exceeding the initial target. We have received a total of 1815 completed questionnaires and have collected 1080 fresh tumour tissue samples and 1582 blood samples. Control recruitment is also complete and a total of 1066 control women that did not have ovarian cancer were recruited. Clinical details have been recorded for all AOCS patients, with clinical follow-up done at 6-monthly intervals: we have primary treatment data, including surgery and chemotherapy details on 99% of cases; and 89% of eligible cases have follow-up to five years post-diagnosis. Thus far only 123 patients (6.5%) have been lost to follow-up, despite that fact that 30-40% of our patients return to regional areas for ongoing treatment.

Relapse disease and collection of ascites: We are collecting ascites and tumour tissue (excess to diagnostic requirements) from women who present with recurrent disease. To date we have collected 337 ascitic fluid samples from 214 patients, with scientific analysis of these cases currently underway.

AOCS resource: AOCS is a resource for both local and international researchers who can apply to access biospecimens and associated data. Materials available include DNA (tumour and germline), RNA, plasma, serum, fresh frozen tissue, formalin-fixed paraffin embedded (FFPE) blocks, Tissue Microarrays (TMA) and extensive clinical and epidemiological data. To date we have approved approximately 70 national and international projects and collaborations, some of which are listed below:

- SWI/SNF mutations in clear cell carcinoma (BC Cancer Agency, Vancouver, Canada)
- International Cancer Genomics Consortium Study (PMCC, Melbourne, Aus)
- HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy (University of British Columbia, Vancouver, Canada)
- Genetic relationships between breast and ovarian cancer: shared chromosomal alterations and response to chemotherapy (Dana Faber Cancer Institute, Boston, USA)
- Quality of life among women with platinum-resistant and platinum-sensitive ovarian cancer who were treated for recurrence: A prospective study (QIMR, Brisbane, Aus)
- Development of a novel xenograft mouse model of high grade epithelial ovarian cancer for analysis of therapeutic manipulation in order to improve outcomes for patients with ovarian cancer (Walter and Eliza Hall Institute, Melbourne, Aus)
- Deep sequencing of 19p13 (National Cancer Institute, Maryland, USA)

Application forms and a complete list of approved projects can be found at http://www.aocstudy.org.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Thorne et al: The kConFab experience – 14 years of biobanking.