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PROFFERED PAPERS

S1-PP1

Incidence of *BRCA1* and *BRCA2* Non-Founder Mutations in Patients of Ashkenazi Jewish Ancestry

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Objectives: It is estimated that 1%–2% of individuals of Ashkenazi Jewish (AJ) ancestry carry one of three pathogenic founder mutations in *BRCA1* and *BRCA2*. Targeted testing for these mutations (*BRCA1* 187delAG and 5385insC, and *BRCA2* 6174delT) is therefore recommended for all AJ breast and ovarian cancer patients, regardless of age of diagnosis or family history. Comprehensive analysis of both genes is recommended for a subset of AJ patients in whom founder mutations are not identified, but estimates of the yield from comprehensive analysis in this population vary widely.

Methods: We sought to establish the proportion of non-founder mutations in AJ patients undergoing clinical testing in our laboratory from January 2006 through August 2013. Analysis included AJ patients for whom: 1) comprehensive testing was ordered as the initial test, or 2) founder mutation testing was ordered with instructions to "reflex" to comprehensive analysis if negative. The latter group was limited to cases where the reflex testing was ordered on the original test request form, and not cancelled for any reason other than the detection of a founder mutation.

Results: The percentage of non-founder mutations detected in these groups was 13% (104/802) and 7.2% (198/2769) respectively. We detected 189 unique non-founder mutations, 76 in *BRCA1* and 113 in *BRCA2*. *BRCA2* 4075delGT was detected in 15 patients. The next most common mutations, found in 7 patients each, were *BRCA1* 5055delG, *BRCA2* 1982delA, and *BRCA2* R3128X.

Conclusions: Non-founder mutations make up between 13% and 7.2% of *BRCA1* and *BRCA2* mutations in patients reporting AJ ancestry. These numbers may represent underestimates if some patients were ascertained for testing based on the identification of a founder mutation in a relative. These numbers suggest that the prevalence of non-founder mutations in AJ individuals may be comparable to the prevalence of *BRCA1*/2 mutations in non-AJ individuals.

PROFFERED PAPERS (CONTINUED)

S1-PP2

BRCA1 Circos: A Visualization Resource for Functional Analysis of Missense Variants

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Objectives: Inactivating germline mutations in the tumour suppressor gene *BRCA1* are associated with a significantly increased risk of developing breast and ovarian cancer. A large number (>1500) of unique *BRCA1* variants have been identified in the population and can be classified as pathogenic, non-pathogenic or variants of unknown significance (VUS). Many VUS are missense variants leading to single amino acid changes. Their impact on protein function cannot be directly inferred from sequence information precluding assessment of their pathogenicity. Thus, functional assays can be used to assess the impact of these VUS. *BRCA1* is a multifunctional protein and different assays have been used to assess the impact of the same variant on different biochemical activities. However, there are no tools that consolidate all the functional data available for these missense variants in one comprehensive and searchable database. Therefore, there is a need for a visualization tool that can be used to analyze and interpret the functional impact of *BRCA1* missense variants.

Methods: To facilitate VUS analysis, we developed a visualization resource that compiles and displays functional data on all documented *BRCA1* missense variants. *BRCA1* Circos is a Web-based visualization tool based on the freely available Circos (http://circos.ca) software package that utilizes PERL programming.

Results: The *BRCA1* Circos Web tool aggregates data from all published *BRCA1* missense variants functional studies, harmonizes their results, and presents various functionalities to search and interpret individual-level functional information for each *BRCA1* missense variant. The current version of *BRCA1* Circos resides at http://lgdfm3.ncifcrf.gov/bic/bic.html and is a comprehensive and easy-to-follow Web resource.

Conclusions: This research visualization tool will serve as a quick one-stop publically available reference for all the *BRCA1* missense variants that have been functionally assessed. It will facilitate meta-analysis of functional data and improve assessment of their pathogenicity.

S1-PP3

Identification, Prediction, and Prioritization of Non-Coding Variants of Uncertain Significance in Heritable Breast/Ovarian Cancer

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Objectives: Deleterious variants are not identified in many heritable breast/ovarian cancer (HBOC) patients, despite linkage and case–control studies suggesting that known predisposing genes harbour mutations. Considering that such variants might reside in unsequenced non-coding gene regions, we are determining complete HBOC gene sequences. We present a unified framework, based on information theory, to predict pathogenic non-coding variants of uncertain significance (VUS) from changes in DNA/RNA sequences bound by regulatory factors.

Methods: Complete gene sequences are captured by solution hybridization, enriching for coding and non-coding variants. Oligo baits covering coding, intronic, and intergenic regions 10kb upstream/downstream of *ATM, BRCA1*, *BRCA2*, *CDH1, CHEK2, PALB2,* and *TP53* were designed and manufactured. Repeat-free and divergent repeat sequences that are effectively single copy were sequenced in 102 high-risk patients without known mutations in these genes. Information theory-based analysis prioritized VUS within binding site sequences recognized by proteins or protein complexes. Information models were generated by entropy minimization of ChIP-seq regions for 32 transcription factors binding within DNASE I hypersensitive domains, and CLIP data from 37 RNA binding proteins in untranslated region (UTR) sequence analyses. The models flag variants that alter information contents of binding sites required for mRNA splicing, transcription factor binding (TFBS), and of proteins interacting with UTRs. Additionally, information models for exon recognition predict relative abundance of normal and mutant splice isoforms.

Results: Information analysis of 15,755 variants highlighted functionally significant VUS. The analysis prioritized 5 splicing, 5 stop-gain mutations, and 4 reading-frame altering exonic insertions/deletions. Additionally, 14 TFBS, and 12 UTR variants affecting protein binding site strength were predicted to alter expression of these genes.

Conclusion: The hybridization enrichment strategy more comprehensively covers non-coding regions in HBOC genes than repeat-masked capture approaches. Information analysis provides a unified framework for prioritizing VUS affecting gene expression, revealing potentially unrecognized mutations in established HBOC genes.

S2-PP1

Identification and Validation of an Anthracycline/Cyclophosphamide–Based Chemotherapy Response Assay in Breast Cancer

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Objectives: There is currently no method routinely employed to predict response to anthracycline/cyclophosphamide–based chemotherapy in the clinic. Loss of the Fanconi anemia/*BRCA* (FA/*BRCA*) DNA-damage response (DDR) pathway occurs in approximately 25% of breast cancer. The objective of this study was to develop an assay to identify patients with dysfunction of the FA/*BRCA* pathway that could inform the selection of effective DNA-damaging agents in the clinic.

Methods: The genetic processes associated with a deficiency in DDR were identified using publicly available gene expression data from 21 FA patients and 11 controls. A DNA-microarray platform optimized for formalin-fixed, paraffin-embedded (FFPE) tissue was used to analyze a *BRCA1/2* mutant enriched cohort of FFPE breast cancer samples. Molecular subgroups within this cohort were defined using unsupervised hierarchical clustering analysis, resulting in identification of a molecular subgroup characterized by the molecular processes modulated in response to dysfunction of the FA/*BRCA* pathway. A 44-gene microarray-based assay was developed to prospectively identify this subgroup.

Results: The assay predicted pathologic complete response versus residual disease following neoadjuvant DNA-damaging chemotherapy (5-fluorouracil, anthracycline, and cyclophosphamide) with an odds ratio of 3.96 (95% CI: 1.67 to 9.41; $p = 0.002$) in a publicly available independent cohort of 203 patients. The assay was also applied to FFPE samples from an independent validation cohort of 191 breast cancer (N0–N1) patients who received adjuvant 5-fluorouracil, epirubicin, and cyclophosphamide treatment. A positive assay result predicted 5-year relapse-free survival with a hazard ratio of 0.37 (95% CI: 0.15 to 0.88; $p = 0.03$) compared to the assay negative population.

Conclusion: This study has identified a molecular subgroup in breast cancer, which can be identified by a 44-gene assay. This assay has been developed and independently validated as a predictor of response and prognosis following anthracycline/cyclophosphamide–based chemotherapy in the neoadjuvant and adjuvant settings. These findings warrant further validation in a prospective clinical study.

PROFFERED PAPERS (CONTINUED)

S2-PP2

Screening Formalin-Fixed and Paraffin-Embedded (FFPE) Archival Tissue for Mutations in *BRCA1/2*— A New Paradigm in Genetic Counselling

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In many families with a history of breast and ovarian cancer suggestive of a germline mutation in the *BRCA1* or *BRCA2* genes, the proband is deceased and accurate cancer risk estimation may be difficult. Previously, it was possible to detect only the Ashkenazi Jewish *BRCA1/2* founder mutations in DNA extracted from archival formalin-fixed paraffin-embedded (FFPE) tissue, but screening *BRCA1/2* for other mutations has so far been unsuccessful. We developed a routine analysis to screen archival FFPE samples for mutations in *BRCA1/2* using HaloPlex target enrichment (Agilent) and next-generation sequencing technology (Illumina) with the purpose of determining whether a deceased relative did harbour a germline *BRCA1/2* mutation or not, to aid family members seeking genetic counselling.

This study included 32 FFPE samples from normal (non-cancer) tissue, 27 samples from women with a known germline *BRCA1/2* mutation and 5 samples from women with no germline *BRCA1/2* mutations. The mutation status (*BRCA1/2* mutation or wild-type) was unknown to the scientific staff performing the analyses. This study focused on examining the relationship between the type and age of FFPE sample, quality and amount of DNA required to get a successful positive or negative result.

A total of 30 HaloPlex libraries were successfully prepped. As expected, larger exon deletions were not detected. However, we managed to correctly report the *BRCA1/2* status of 25 FFPE samples (83.3%) despite differences in quality and archival age.

Today, this analysis is being offered to families seeking genetic counselling at Department of Clinical Genetics at Vejle Hospital, Denmark. So far, 14 FFPE samples from deceased have been analyzed and resulted in the detection of a pathogenic *BRCA1/2* mutation in 2 cases. The relatives of the deceased have been informed and have received genetic counselling and predictive testing.

S3-PP1

A Novel *BRCA1*-mRNA Splicing Complex Is Required for Efficient DNA Repair and Maintenance of Genomic Stability

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Mutations within *BRCA1* predispose carriers to a high risk of breast and ovarian cancers. *BRCA1* functions primarily to maintain genomic stability through the assembly of multiple protein complexes involved in DNA repair, cell cycle arrest, and transcriptional regulation. Here we report the identification of a novel DNA damage–induced *BRCA1* complex containing BCLAF1, U2AF65, and other key components of the splicing machinery. In response to DNA damage, this complex regulates pre-mRNA splicing of a number of genes involved in DNA damage signalling and repair, thereby promoting the stability of these transcripts/proteins.

Further, we show that abrogation of this complex results in sensitivity to DNA damage, defective DNA repair and genomic instability. Interestingly, mutations in a number of proteins found within the *BRCA1*-mRNA splicing complex have recently been identified in numerous cancer types. These data suggest that regulation of mRNA splicing by the *BRCA1*-mRNA splicing complex plays an important role in the cellular response to DNA damage and maintenance of genomic stability.

S2-PP3

Germline and Somatic *SMARCA4* Mutations Characterize Small-Cell Carcinoma of the Ovary, Hypercalcemic Type

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Background: Small-cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is an aggressive tumour and the most common type of undifferentiated ovarian malignancy presenting at less than 40 years of age. Its cause and histogenesis remain unknown.

Methods: We sequenced the exomes of individuals from three familial cases of SCCOHT. Subsequently, we used whole exome sequencing, Sanger sequencing, and immunohistochemistry to analyze germline and tumour DNA from 3 additional familial cases, 35 nonfamilial cases, and 1 SCCOHT cell line (BIN-67). All cases were reviewed by 2 reference pathologists.

Results: DNA sequencing revealed likely deleterious germline mutations in the chromatin remodelling gene *SMARCA4* in all four familial cases of SCCOHT where DNA was available. This was accompanied by either a somatic mutation or loss of the wild-type allele in the tumour. BIN-67 contained bi-allelic deleterious mutations in *SMARCA4*. Sequencing of 24 nonfamilial pathologically-confirmed SCCOHT cases revealed at least one germline or somatic likely deleterious *SMARCA4* mutation in 22 cases. Immunohistochemical analyses of these and an additional 11 tumours showed loss of Brg1 protein in 38/40 cases. Loss of Brg1 was not observed in any ovarian tumour that is known to morphologically mimic SCCOHT.

Implications: Our findings identify alterations in *SMARCA4* as a major cause of SCCOHT, which could pave the way for genetic counselling and new treatment approaches. Brg1 immunohistochemistry will be useful in helping to diagnose this unusual tumour.

PROFFERED PAPERS (CONTINUED)

S3-PP2

BRCA1 Haploinsufficiency Compromises the Repair of Stalled Replication Forks and Increases the Risk of Genomic Instability

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BRCA1 (B1) is a breast and ovarian cancer suppressor that maintains genome integrity by engaging in multiple cellular processes, including the repair of DNA damage. We and others have recently shown that B1 exhibits a new DNA damage repair function—that is, repair of stalled replication forks (SRFS). Stalled forks, when not resolved, lead to mutations, or collapse into double strand breaks (DSBS). Both outcomes result in what is commonly referred to as replication stress (RS), which, when chronic, is a driving force behind cancer development.

To ask whether repair of SRFS is defective in primary, ostensibly normal breast epithelial cells in B1 mutation carriers, and whether such a state of haploinsufficiency results in the types of genomic changes that lead to cancer, we have now generated 18 primary fibroblast lines from skin punch biopsies and 7 primary mammary epithelial cell (MEC) lines from prophylactic mastectomies performed on B1 mutation-carrying women. This collection includes $n = 23$ different B1 mutations, which, collectively, span almost the entire B1 gene. B1+/+ control MECS were derived from mammary tissue collected during reduction mammoplasty. Control fibroblasts were derived from skin punch biopsies obtained from women without a B1 mutation.

Our current data show that while all of our B1+/– fibroblasts and MECS behaved normally for other *BRCA1* functions such as spindle pole formation and centrosome maintenance, we found that these cells were defective in repair of SRFS after exposure to replication-stalling agents such as hydroxyurea and ultraviolet radiation. More specifically, we observed that a) p-RPA32–coated-SSDNA generation, a prerequisite for G2 checkpoint activation and the repair of a stalled fork, was defective, and b) inefficient SRF in B1+/– fibroblasts and MECS resulted in an increase in collapsed forks and increased DNA breaks—that is, signs of ongoing RS. The defects in RPA loading in B1+/– cells were rescued by reconstituting the cells with lentivirus-encoded wild-type B1 CDNA. In addition, when compared to wild-type B1+/+ cells, B1+/– cells in a FACS-based "color-coded assay," revealed heightened sensitivity to stalled fork–inducing agents. No evidence of an homologous recombination-based double-strand break repair defect nor supersensitivity to PARP inhibitors was observed.

Based on these data, we hypothesize that SRF is a B1 gatekeeper function that is defective early on in the life of mammary epithelial progenitor cells of B1+/– women. Moreover, we speculate that this defect, as a potential source of chronic RS, represents a key functional abnormality in the pathway leading to breast cancer in these individuals.

S3-PP3

Chemotherapy Depletes Intratumoural PARP1 Protein in Ovarian Cancer Patients

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Ovarian cancer is the deadliest gynecologic cancer and is responsible for the death of 1750 Canadian women each year. Early detection and chemotherapy treatment have not improved the 5-year overall survival for high-grade serous ovarian cancer, which remains lower than 30%. To counter this drawback, identification of key biomarkers for one or a combination of specific antitumour drugs seems essential. In the last 5 years, clinical trials using PARP inhibitors gave promising results in ovarian cancer patients with *BRCA1* mutation.

Objective and Method: *In vitro* experiments made in cell lines with silenced *BRCA1* showed that patients without a *BRCA1* mutation but with *BRCA1* silenced gene expression could potentially respond to PARP inhibitors. To evaluate if these findings could translate in patients, we screened tumours from 48 patients with serous adenocarcinoma for *BRCA1* and PARP1 protein expression by Western blot.

Results: We observed two major findings: first, 77% of ovarian cancer patients do not express *BRCA1* in the tumours; second, tumours isolated from patients treated with chemotherapy have no PARP1 expressed in them.

Conclusions: These are significant, first because the proportion of patients without *BRCA1* who could benefit from PARP inhibitor treatment is close to 80%. Second, patients with no PARP1 expression cannot possibly respond to PARP inhibition. This observation was further confirmed using matched cancerous tissues isolated from the same patient before and after chemotherapy. These data indicate that chemotherapy might directly affect the level of PARP1 in the tumours. Finally, our data suggest that sequential administration of PARP inhibitor followed by chemotherapy could be more effective than the reverse order, which is currently the standard order of drug administration to patients in clinical trials. We propose that the change in drug administration order can significantly increase the number of patients who respond to PARP inhibitors combined with classic chemotherapy.

S4-PP1

The Impact of Oophorectomy on Survival after Breast Cancer in *BRCA1* and *BRCA2* Mutation Carriers

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Purpose: To estimate the impact of oophorectomy on survival from breast cancer for women with a *BRCA1* or *BRCA2* mutation.

Patients and Methods: 760 women with stage I or stage II breast cancer and a *BRCA1* or *BRCA2* mutation, between the ages 25 and 65, were followed for up to 20 years from diagnosis. The impact of oophorectomy on survival was evaluated in a Cox proportional hazards analysis.

Results: Of the 760 women, 455 had an oophorectomy, either prior to or after the diagnosis of breast cancer. The 20-year survival for the entire patient cohort was 74.3%. The unadjusted hazard ratio for death associated with oophorectomy was 0.62 (95% CI: 0.42 to 0.90; $p = 0.01$) and the adjusted hazard ratio was 0.66 (95% CI: 0.42 to 1.02; $p = 0.06$). The hazard ratio was 0.59 (95% CI: 0.34 to 1.01; $p = 0.05$) for *BRCA1* carriers and 0.81 (95% CI: 0.35 to 1.85; $p = 0.61$) for *BRCA2* carriers. The adjusted hazard ratio was 0.77 (95% CI: 47 to 1.28; $p = 0.29$) for women diagnosed under age 50 and 0.38 (95% CI: 0.12 to 1.15; $p = 0.09$) for women diagnosed over age 50. The hazard ratio was 1.21 (95% CI: 0.55 to 2.67; $p = 0.65$) for women with estrogen receptor–positive breast cancer and 0.27 (95% CI: 0.11 to 0.67; $p = 0.005$) for women with estrogen receptor–negative breast cancer.

Conclusion: Oophorectomy is associated with a decrease in mortality in women with early-stage breast cancer and a *BRCA1* mutation. Women with estrogen receptor–negative breast cancer and a *BRCA1* mutation should consider oophorectomy shortly after diagnosis as part of their treatment plan.

PROFFERED PAPERS (CONTINUED)

S4-PP2

Effect of Germline *BRCA* Mutations on Response to Chemotherapy and Outcome of Recurrent Ovarian Cancer

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Objective: Treatment of recurrent epithelial ovarian cancer (rEOC) remains a major challenge because of development of platinum resistance. Recent evidence has suggested that *BRCA* mutation carriers may have improved outcome to treatment compared to patients with non-hereditary (NH) disease. We summarized the experience following chemotherapy treatment of rEOC in one institution and compared the outcome in *BRCA* mutation carriers versus NH subsets.

Methods: We retrospectively analyzed 256 patient records with rEOC who were treated with second-, third-, and fourth-line treatment with the usual sequential regimens consisting of either pegylated liposomal doxorubicin (PLD), taxanes, gemcitabine, or topotecan (alone or in combination with platinum) between 2002 and 2012 at our institution and compared the outcome in *BRCA* mutation carriers with that of patients with NH disease.

Results: *BRCA* mutation carriers treated with PLD (with or without platinum), gemcitabine plus platinum, or platinum alone had improved progression-free survival (PFS) and a lower risk for disease progression (adjusted for age, line of treatment and platinum sensitivity) compared with patients with NH disease. By contrast, treatment with taxanes (with or without platinum) or topotecan led to similar PFS in *BRCA* mutation carriers and in patients with NH disease. Under all treatment regimens, *BRCA* mutation carriers showed improved overall survival after adjusting for age, line of treatment, and platinum sensitivity.

Conclusions: This single-institution experience provides indications of an enhanced benefit in PFS for *BRCA* mutation carriers compared to patients with NH disease across a number of drug regimens (PLD, platinum, or gemcitabine plus platinum) regardless of platinum sensitivity and line of therapy.

S4-PP3

MRI Volumetric Analysis of Breast Fibroglandular Tissue to Assess Risk of the Spared Nipple in *BRCA 1* and *BRCA2* Mutation Carriers

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Objectives: Prophylactic nipple areolar complex (NAC)–sparing mastectomy (NSM) in *BRCA1/2* mutation carriers is controversial over concern that residual fibroglandular tissue (FGT) with malignant potential remains in the spared NAC. The objective of this study was to model the volume of FGT in the NAC at a standard retroareolar margin (5 mm) and to examine the change in this amount with a greater retroareolar margin or areola-sparing technique.

Methods: A segmentation protocol was applied to breast MRIs from 105 *BRCA1/2* patients to quantify volumes of FGT for total breast and NAC. The proportion of FGT in the NAC relative to the breast was calculated as the primary outcome and was compared for 5 mm versus 10 mm retroareolar depths. The proportion of FGT in the areola was compared with the NAC.

Results: At 5 mm retroareolar thickness, residual NAC FGT comprised 1.3% of the total breast FGT. This amount was not significantly greater than the proportion in the areola ($p = 0.3$, $d = 0.1$). Increasing the retroareolar thickness to 10 mm led to a statistically and possibly clinically significant increase in the amount of NAC FGT ($p < 0.001$, d = 1.1).

Conclusions: The proportion of FGT remaining in the spared NAC with a 5-mm margin is extremely small, suggesting that leaving the entire NAC would create very little added risk. Doubling the retroareolar margin may translate into a clinically meaningful increase. Overall, our findings support the safety of the current trend toward increased rates of prophylactic NSM performed in this high-risk population.

S5-PP1

Effect of *BRCA* Mutations on Biochemical Relapse and Survival After Treatment for Localized Prostate Cancer

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Background: Biochemical relapse after local treatment for prostate cancer (PCa) indicates recurrent disease and is associated with shorter cause-specific survival (CSS). Germline *BRCA* mutations are associated with worse PCa outcomes. In this study, we analyzed biochemical progression-free survival (bPFS) after conventional treatments for localized PCa in a cohort of *BRCA* patients. Currently, *BRCA1/2* carriers are treated with the same protocols used for non-carriers.

Methods: Each *BRCA* carrier (10 *BRCA1* and 34 *BRCA2*) treated with radical prostatectomy (RP) or external-beam radiotherapy (RT) was matched with 3 non-carriers (NCs) by age at diagnosis (±5 years), TNM stage, Gleason score, presenting PSA, local treatment (RT or RP), androgen-deprivation therapy (ADT), and year of treatment (±5 years). Biochemical failure was reviewed according to ASTRO and NCCN criteria. The Kaplan–Meier method and a multivariate Cox regression model adjusted by matching factors were employed.

Results: 176 Patients were included. Median follow-up was 97 months. Median age at diagnosis was 58.5 years (43–75). 80 Patients received RT (16 *BRCA2*, 4 *BRCA1*, 60 NC) and 85% also received ADT ≥ 6 months. 96 Patients underwent RP (18 *BRCA2*, 6 *BRCA1*, 72 NC). Following RT treatment, 5-year CSS was 96% in NCs and 47% in *BRCA* carriers ($p = 2\times10^{-5}$), whilst no difference was seen after RP (5-year CSS was 98.5% in NCs vs. 93.3% in *BRCA*). Five years after RT, 74% NC and 24% *BRCA* were free from biochemical relapse ($p = 0.002$). The adjusted MVA confirmed the independent prognostic value of *BRCA* status for bPFS and CSS. No difference was observed in 5-year bPFS between *BRCA* carriers and NCs treated with RP (52% vs. 66%, $p = 0.346$).

Conclusions: Our results suggest that *BRCA* carriers have worse local disease control than NCs when conventionally treated with RT. No differences in bPFS were observed in patients treated with RP. These results may have implications for tailoring clinical management in these patients.

S5-PP2

The Impact of a *BRCA2* Mutation on Mortality from Screen-Detected Prostate Cancer

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Men with a *BRCA2* mutation face an increased risk of prostate cancer and are advised to undergo annual screening with a PSA test. These cancers tend to have an aggressive nature, and it has not yet been demonstrated that regular screening of *BRCA2* carriers is associated with improved survival. We identified 4187 men who underwent a prostate cancer biopsy for an elevated PSA or an abnormal digital rectal examination between 1998 and 2010. We screened the *BRCA2* gene in its entirety for mutations and we followed the men for death from prostate cancer until December 2012. The 12-year prostate-cancer specific survival rate was 94.3% for men without a mutation and was 61.8% for men with a mutation ($p < 10^{-4}$; log rank test). After adjustment for age at diagnosis, grade, and PSA level, the presence of a *BRCA2* mutation was associated with a 3-fold increase in prostate cancer mortality (HR: 3.48; 95% CI: 1.58 to 7.67).

PROFFERED PAPERS (CONTINUED)

S5-PP3

Contribution of Known and Novel *BRCA*-Mediated DNA Repair Pathway Genes to Pancreatic Cancer Susceptibility

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Although 10% of pancreatic adenocarcinoma (PC) cases cluster in families, the genetic basis underlying most familial PC (FPC) cases remains unknown. Mutations in the *BRCA*-pathway genes (*BRCA1/2*, *PALB2*) are rare causes of PC, but may have a more significant role in French-Canadians (FCS), a population enriched with founder mutations in these genes. We have hypothesized that 1) mutations in the *BRCA*-pathway genes account for 5% of PC in FCS and, 2) that FPC cases without mutations in known PC susceptibility genes have causative mutations in other tumour suppressor genes.

In a prospective clinic-based study, we screened unselected incident PC cases with FC ancestry ($n = 52$) for the common FC founder mutations ($n = 20$) and uncovered *PALB2* and *BRCA2* founder mutations in 2 kindreds. Additionally, we identified a novel loss-of-function *BRCA2* mutation in a third FC family. Since the role of *PALB2* as a PC susceptibility gene is not well established, we provide supporting evidence by confirming mutation segregation with disease, and loss of the wild-type allele in the corresponding tumours. Of note, both *BRCA2* carriers were treated with platinum-based chemotherapy, targeting DNA repair defects in their tumours, and demonstrated marked tumour responses.

To search for novel genetic causes of PC, we identified 99 high-risk families, including familial and young-onset PC cases, collected through the Quebec and Ontario Pancreas Cancer studies. We employed exome sequencing to interrogate all of the protein-coding regions of the genome in these high-risk cases, as well as in 13 PC-affected relatives. We identified 9 putative FPC genes with rare, loss-of-function variants among multiple high-risk families. Two of these candidate genes are involved in the *BRCA*-mediated DNA repair pathway.

Our data suggest that *BRCA*-pathway mutations may contribute significantly to PC susceptibility in FCS. As well, we have identified 9 novel FPC candidate genes, including 2 genes involved in the *BRCA* pathway.

S6-PP1

Access to Genetic Counselling and *BRCA* Testing Among a Population-Based Sample of Young Black Women

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Background: Concerns about the potential for genomic advances to increase health disparities have been raised. Within the United States, although any physician may order *BRCA* testing, conduct of genetic counselling (GC) prior to *BRCA* genetic testing (GT) remains the "gold standard," endorsed by many professional organizations. Thus it is important to assess GC referral/uptake and GT access in minority populations.

Methods: Black women diagnosed with invasive breast cancer (BC) < age 50 in 2009–2012 were recruited through the Florida State Cancer Registry 6–18 months following diagnosis and completed a baseline questionnaire. Summary statistics and logistic regression were used to examine associations between demographic variables and access to GC and GT at enrollment.

Results: All 287 participants met national criteria for referral for GC, yet only 95 (33%) were referred, of which 49 attended (44 of whom underwent GT). An additional 52 underwent GT (including 15 referred for GC but did not attend). Additionally, there were 150 (52%) who were never offered GC or *BRCA* testing. The only variable associated with having GC was referral by a health care provider. Variables positively associated with receiving GT included GC, private health insurance, younger age, and higher household income. Marital status and having had children were not associated with undergoing GT.

Conclusions: The population-based study design provides a more accurate estimate of access compared to other designs and results suggest efforts are needed to improve access to GC and GT. Our results indicate that the majority of participants were not referred to or attended GC or offered GT. Furthermore, referrals by health care providers were a key determinant in GC access. Even among an ethnic minority population, disparities in access to GT exist and they appear to be due, at least in part, to socioeconomic factors and referral patterns of physicians.

S6-PP2

Breast Cancer Risk Estimation Methods in a Clinic-Based Cohort of *BRCA1/2* Mutation Carriers

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Objectives: Estimates of the cumulative lifetime risk (CLTR) of breast cancer by age 70 in carriers of a pathogenic *BRCA1/2* mutation (*"BRCA1/2* carriers") range between 27% and 88%. As these estimates are based on retrospective studies, several methodologies are applied to correct for related selection bias. The objective of this study was to analyze the effects of these correction methods on the estimated CLTRS in a clinic-based population of *BRCA1/2* carriers.

Methods: A literature search was conducted to identify methods estimating the risk of breast cancer in *BRCA1/2* carriers. Identified methods were applied to our Family Cancer Clinic database consisting of data on 367 extended *BRCA1/2* families. The point estimates of the CLTR and their standard errors yielded by these methods were compared using descriptive statistics, and also compared to recent prospective CLTRS from the EMBRACE study.

Results: We identified 17 studies, including several variations of the Kaplan–Meier analysis. Survival analyses (including all carriers, with bias correction by excluding index cases, including all untested first-degree relatives, including a proportion of untested first-degree relatives) yielded estimates by age 70 of 42%–69% [standard error (SE): 2.0%–3.8%] for *BRCA1* and 43%–74% (SE: 2.5%–6.0%) for *BRCA2* carriers. Including only incident cases increased the risks to 81%–87% (SE: 5.3%–6.2%) and 77%–89% (SES: 10%) respectively. When compared to the prospective EMBRACE estimates, the first CLTRS were not significantly different, whereas the incident case analysis led to point estimates outside the 95% confidence intervals.

Conclusions: Much of the variation in the breast cancer CLTRS in retrospectively ascertained *BRCA1/2* carriers is due to the chosen methods for correction for selection bias. The most restrictive correction, including only incident cases, probably yields excessively high CLTRS for *BRCA1/2* carriers. In a follow-up study, the effect of modified segregation analysis on the breast cancer CLTRS will be assessed in these families.

S6-PP3

Kintalk.org: Helping Families Communicate Their Genetic Information

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Background: The UCSF Cancer Risk Program provides genetic counselling and testing to individuals and families with hereditary breast and ovarian cancer syndrome (HBOC), which is an inherited cancer predisposition due to mutations in the *BRCA1* and *BRCA2* genes. Recommendations are made for our patients with HBOC to share their genetic risk information with their family members. Unfortunately, studies have shown that some families don't share their genetic information due to barriers like a lack of family communication and family education. To overcome the many barriers families may have with sharing genetic information we have developed Kintalk.org, a novel family communication Web site that will allow families to securely share their genetic information and educate family members. Kintalk was originally created for families with Lynch syndrome (LS) but has recently branched out to include an interactive platform for families with HBOC.

Methods: Kintalk's secure portal allows patients to upload and share genetic information with at-risk relatives through an e-mail invitation the patient generates in Kintalk. Patients can track their invitations to ensure that their relatives have received their important genetic information. The nonsecure portal provides the general public with educational information on HBOC. Kintalk members have access and participate in the Kintalk Chat Feed where individuals with LS and HBOC can post questions and share experiences.

Results: As of January 2014, UCSF will be assessing the success of Kintalk for increasing patient's understanding of HBOC and communication in families with HBOC. We will be tracking the number of members, how many Kintalk members upload and share their genetic information with relatives, how many at-risk family members access the Web site and undergo genetic counselling and testing for both HBOC and LS.

Conclusion: Kintalk will allow patients to learn more about LS and HBOC, share their genetic information, and provide the educational material family members need to understand LS and HBOC and undergo genetic testing. By tracking the sharing of information among family members we will be able to determine if this method of communication increases the number of individuals who are informed about LS or HBOC in their family and undergo genetic testing.

PROFFERED PAPERS (CONTINUED)

S9-PP1

Bi-allelic *BRCA1* Mutations Identified through Whole-Exome Sequencing in a Patient with Intellectual Disability, Short Stature, Microcephaly, and Early-Onset Breast Cancer

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We identified bi-allelic *BRCA1* mutations in a patient presenting with mild intellectual disability, short stature, microcephaly, eczema, sparse hair, and facial features consistent with Dubowitz syndrome (DS; MIM 223370). She also had proximally placed thumbs, 2–3 toe syndactyly, an enlarged kidney, and hyper- and hypopigmented lesions. She developed breast cancer at age 23. She was enrolled in a whole-exome sequencing (WES) project through FORGE Canada (Finding of Rare Disease Genes in Canada) as part of a DS cohort in order to identify the genetic basis of this disorder. The results of WES showed bi-allelic mutations in *BRCA1* that were validated by Sanger sequencing and were inherited from each parent. No other compelling variants were identified to account for the DS phenotype. Both of the mutations are pathogenic according to ACMG criteria (c.4954C>T; p.Arg1652Trp, c.453_456del; p.Ser151Argfs*35). DNA breakage studies demonstrated increased sensitivity to ionizing radiation and further functional studies are underway.

This is the second patient to our knowledge to be reported with bi-allelic *BRCA1* mutations. The first patient was reported to have developmental delay, short stature, and microcephaly with ovarian cancer at 28 years of age. Although previously thought to be embryonically lethal, these two patients together suggest that there is a risk for couples who both carry a pathogenic *BRCA1* mutation of having a child with two mutations in *BRCA1*. This patient also demonstrates the importance of looking at all genes in a WES study, including those thought to be relevant to adult onset disorders, and highlights the overlap between incidental and diagnostic findings. In children with unexplained intellectual disability, microcephaly, and dysmorphic features, *BRCA1* mutations should be considered, and identification of a larger cohort of patients will increase the understanding of this Fanconi anemia–like phenotype.

S9-PP2

PALB2 Is a High-Risk Breast Cancer Susceptibility Gene: A Kin-Cohort Analysis of 154 Families

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Background: Germline mutations in *PALB2* are known to predispose to breast cancer (BC). However the BC risk conferred by *PALB2* mutations is uncertain.

Method: We undertook a multicentre ($n = 17$) study and analyzed 154 families including 362 mutation carriers with deleterious truncating or splice *PALB2* mutations. Age-specific breast cancer risks were estimated using a modified segregation analysis approach using models that allowed for the *PALB2* mutation effect and also modelled the residual familial aggregation of BC.

Results: Under the most parsimonious model, the average cumulative risk of BC was estimated to be 14% (95% CI: 9% to 20%) by age 50 and 35% (95% CI: 26% to 46%) by age 70. However, there was significant evidence that BC risks vary by other familial risk factors ($p = 0.035$). Overall, the average risk in female mutation carriers by age 70 ranged from 33% (95% CI: 25% to 44%) in a *PALB2* carrier with no family history to 58% (95% CI: 50% to 66%) in a carrier with 2 first-degree relatives diagnosed with BC by age 50.

Conclusion: We found that *PALB2* mutations are second only to *BRCA1*/*BRCA2*/*TP53* mutations as a cause of hereditary breast cancer and that the risk is higher than previously recognized. Risk estimates should take family history into account, and we recommend that those at highest risk should be managed as for *BRCA1*/*A2* mutation carriers, though ovarian cancer screening/prevention may not be required.

S9-PP3

Identification and Validation of Familial Breast Cancer Susceptibility Genes Using Targeted Sequencing

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Objectives: The genetic cause of the majority of multiple-case breast cancer families remains unresolved. Next-generation sequencing has emerged as an efficient strategy for identifying predisposing mutations in individuals with inherited cancer. 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 more than 200 exomes show that, on average, each individual carries 30–50 protein truncating mutations and 300–400 rare non-synonymous variants. Heterogeneity among our exome data strongly suggest that numerous moderate penetrance genes remain to be discovered, with each gene individually accounting for only a small fraction of families (~0.5%). This scenario marks validation of candidate breast cancer predisposing genes in large case–control studies as the rate-limiting step in resolving the missing heritability of breast cancer. The aim of this study is to screen genes that are recurrently mutated among our exome data in a larger cohort of cases and controls to assess the prevalence of inactivating mutations that may be associated with breast cancer risk.

Methods: We are currently using the Agilent HaloPlex Target Enrichment System to screen the coding regions of 168 genes in 1000 *BRCA1*/*2* mutation-negative familial breast cancer cases and 1000 cancer-naïve controls.

Results: To date, our interim analysis has identified 23 genes which carry truncating mutations in multiple breast cancer families and do not have corresponding truncating mutations among controls, therefore representing interesting candidate genes for further investigation. Among these genes are established breast cancer susceptibility gene *PALB2,* DNA repair genes *ATR, PARP4, TDP1,* and GWAS SNP–associated gene *TOX3*.

S10-PP1

Clinical Impact of a Multiple Gene Sequencing Panel for Hereditary Breast Cancer Families

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Objective: Panels assaying multiple hereditary cancer risk genes are entering clinical use, however little is known about their yield or effect on clinical management of patients. We performed a pilot study on 413 patients to assess the utility of such a panel.

Methods: Germline DNA samples were collected from adult female patients referred to the Stanford Clinical Cancer Genetics Program for assessment of hereditary breast and ovarian cancer risk. Two thirds had cancer and 14% had mutations in *BRCA1*/*2* detected by previous traditional testing. These patients were re-sequenced for *BRCA1*/*2* plus 25 additional genes using methods that detect both sequence changes and copy-number deletions/duplications. Pathogenicity of variants was assessed following American College of Medical Genetics criteria. Cases were evaluated for return of these results; selected variants were confirmed and patients invited for additional counselling.

Results: In an initial cohort of 170 patients, 18 *BRCA1*/*2* negative individuals were found to have potentially pathogenic variants in other genes including *CDH1, MLH1, MSH2, MSH6, PMS2, PALB2,* and others. The validation cohort of 243 patients showed a similar 10% rate. Variants of unknown significance in non-*BRCA1*/*2* genes were common. 13 Patients from the first cohort were provided additional recommendations, and in some specific cases cancers may have been prevented or detected earlier had the expanded panel been available for use in these patients initially. Clinical follow-up for the second cohort is ongoing.

Conclusion: Use of an expanded panel, with full sequencing of all genes and full del/dup analysis, increased diagnostic yield approximately 50% with the caveat that some lower penetrance genes and additional variants of unknown significance were detected. Communication of this information was found to be feasible for the clinic, well tolerated by the patients, and of medical value.

PROFFERED PAPERS (CONTINUED)

S10-PP2

Identifying the Missing Genetic Risk in Ovarian Cancer

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Germline *BRCA1/2* mutations can strongly influence the response of women with ovarian cancer (OC) to therapy. Identifying genetic risk in family members is the most successful available preventive strategy in OC, through targeted surgical intervention. However, *BRCA1* or *BRCA2* mutations account for only ~40% of the heritable fraction. Our aim is to identify additional risk loci, with an emphasis on rare, moderate penetrance alleles in individual families. Identifying the full repertoire of OC predisposition genes will have a major and immediate impact on how family members are managed and may also be useful on a population basis (in combination with low penetrance alleles to tailor screening and prophylactic interventions). Defining genetic risk loci may also help to pinpoint somatic driver events in sporadic OC suitable for tailored therapeutic strategies.

We are using a family-based whole-exome sequencing to screen women with a personal and/or strong family history of OC but are negative for known pathogenic mutations (OvCaX families). We have whole-exome data from index cases from 20 OvCaX families. We are currently identifying candidate genes that can progress to a validation phase by firstly prioritizing those with overtly deleterious mutations (on average these individuals harbour 15–20 such mutations). We have also derived germline variant data for OC patients sequenced as part of The Cancer Genome Atlas (TCGA), and this will be used to further prioritize candidates based on the frequency of germline and somatic mutations in the TCGA cases. Access to detailed pedigree, tumour pathology information and DNA from family members through the Variants in Practice (VIP) study and a national collaboration of Australian familial cancer centres (the ICCon Partnership) will allow us to examine co-segregation of candidate OC predisposition genes.

S10-PP3

Common Genomic Variants and the Risk of Breast Cancer in *BRCA1* and *BRCA2* Mutation Carriers: Evidence for a Broad Modifying Effect

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Background: Large numbers of common genomic variants, significantly associated with breast cancer risk have been identified through breast cancer GWAS in both the general population and in *BRCA1* and *BRCA2* carriers. Only a limited number of the described variants are common to both groups. We examined the extent of risk modification in mutation carriers from a large panel of recently described breast cancer associated variants identified in population-based GWAS.

Methods: SNP genotypes from two Australian cohorts [KConFab and the Variants in Practice (VIP) study] were combined for the analysis. 1362 mutation carriers [830 *BRCA1*, 533 *BRCA2*, 868 (63%) affected] and 897 population controls were genotyped for >70 SNPs identified in published GWAS with 99% genotyping success. Individual polygenic risk scores (PRSS) were calculated for each case based on published ORS.

Results: Common genomic variants made a significant contribution to breast cancer risk in the context of a *BRCA1* or *BRCA2* mutation. The risk of breast cancer, measured by the PRS, was increased in the group as a whole, reflecting ascertainment. After adjustment for the familial background, polygenic risk was significantly higher in women affected by breast cancer than their unaffected relatives (*BRCA1* PRS 0.16 vs. 0.0, $p = 0.001$; *BRCA2* 0.14 vs. 0.0, $p = 0.009$). The modifying effect was evident even after exclusion of all variants reported to date as modifiers in CIMBA. Rate-based analysis found significant differences in penetrance between the high and low quartiles of polygenic risk. For *BRCA1* carriers, the average time to first breast cancer was 46.9 vs. 54.8 years ($p = 0.03$) in the 25% at highest versus lowest polygenic risk and 49.8 vs. 58.2 years ($p = 0.005$) for *BRCA2* carriers.

Conclusions: In this cohort of *BRCA1/2* carriers the combined effect of the common variants associated with breast cancer in the general population strongly influenced the penetrance of breast cancer.

POSTERS

BRCA1/2 MUTATIONS, VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE, AND DATABASES

P001

Risk Management Options Elected by Women after Testing Positive for a *BRCA1* or *BRCA2* Variant of Unknown Significance Mutation

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Objectives: To describe and compare cancer risk–reducing behaviors in women with *BRCA* mutations of unknown significance to women with known deleterious mutations.

Methods: Women who tested positive for a *BRCA* mutation from 1995–2012 were identified from a community-based integrated health system in Northern California. Exclusion criteria included loss of membership or death within 1 year of testing, prior ovarian cancer, or prior bilateral salpingo-oophorectomy. A retrospective chart review using the electronic medical record was performed. Primary outcomes were rate of risk reducing mastectomy (RRM) and salpingo-oophorectomy (RRSO). Utilization of ovarian cancer risk–reduction strategies was compared with breast cancer risk.

Results: The mean age of the 69 VUS carriers was 50 years vs. 47 years for the 305 women with deleterious mutations. VUS women were followed for a median of 69 months from testing. Of the VUS carriers, 30% underwent RRSO, and 11% underwent RRM compared to 74% and 44% for women with deleterious mutations. Women with a deleterious mutation were more likely to undergo RRSO than women with a VUS (OR: 6.4). Women with a deleterious mutation were more likely to undergo cancer surveillance in the first year after testing than VUS women, 43% vs. 39% for mammogram (OR: 2.1), 35% vs. 15% for MRI (OR: 6.0), 47% vs. 18% for CA125 (OR: 7.7) and 45% vs. 26% for TVUS (OR: 5.0). During follow-up, 54% of VUS mutations were reclassified after a median of 39 months, longer than the median time to RRSO (18.6 months) or RRM (20.1 months).

Conclusions: Uptake of risk-reducing surgery and breast and ovarian cancer surveillance strategies among women with VUS is lower than that of women with known deleterious mutations. Given the prognostic uncertainty and high rate of reclassification for VUS carriers, it may be best to direct efforts toward improving surveillance and considering salpingectomy in this group of women.

P002

A *BRCA2* Missense Mutation Resulting in Mis-splicing and Nonsense-Mediated Decay in a Family Presenting with Premenopausal Breast and Serous Ovarian Cancer

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A hereditary predisposition to breast or ovarian cancer (HBOC) can segregate in an autosomal dominant fashion with mutations affecting either the *BRCA1* or *BRCA2* genes. A majority of obviously deleterious mutations in these two genes consists of nonsense mutations, small insertion/deletion frame-shifting mutations, or mutations affecting the canonical splice sites, which can result in a truncated protein. However, intronic mutations outside the canonical splice sites, or exonic coding sequence variants that may or may not lead to a deleterious amino acid substitution, can cause deleterious mis-splicing resulting in a predisposition to HBOC-related disease.

We present a case report involving a family where the proband had presented with premenopausal breast cancer at age 44 years, and whose sibling had previously been diagnosed with serous ovarian cancer. DHPLC/MLPA followed by targeted direct sequence analysis of leukocyte-derived, PCR-amplified, genomic DNA obtained from the proband, and encompassing the entire coding sequence of both *BRCA1* and *BRCA2* genes, revealed no obviously damaging mutation in either of these two genes, but did demonstrate a novel *BRCA2* coding sequence variant (*BRCA2*:c.7435G>T) indicating an amino acid substitution (*BRCA2*:p.Asp2479Tyr) predicted by *in silico* analysis to be damaging by both the SIFT2 and Polyphen3 algorithms. Further *in silico* analysis using the splice site prediction ASSEDA1 algorithm suggested that this variant would completely eliminate the binding affinity of the contiguous splice donor site (Ri=2.4>–1.0).

Analysis of CDNA by RT-PCR, using leukocyte-derived MRNA obtained from both the proband, as well as from her daughter who carried the same mutation, confirmed loss of the *BRCA2*:c.7435T allele, consistent with Nonsense Mediated Decay4 of the mis-spliced mutant allele.

References:

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BRCA1/2 MUTATIONS, VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE, AND DATABASES (CONTINUED)

P003

Clinical Presentation of *BRCA1* and *BRCA2* Double Heterozygous Mutation Carriers

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Objectives: Hereditary breast and ovarian cancer (HBOC) syndrome is an autosomal dominant condition caused by a mutation in either the *BRCA1* or *BRCA2* gene. A mutation in either *BRCA1* or *BRCA2* greatly increases an individual's risk of developing breast and/or ovarian cancer. On occasion, individuals are observed to carry both a *BRCA1* and a *BRCA2* mutation. There is little in the literature regarding the clinical presentation of double *BRCA1* and *BRCA2* heterozygous individuals. This study compares the clinical presentation of single *BRCA1* and *BRCA2* mutation carriers to double heterozygote *BRCA1* and *BRCA2* mutation carriers.

Methods: A retrospective review of patients' personal history of cancer was performed on patients identified with both a *BRCA1* and *BRCA2* deleterious mutation analyzed in our laboratory from January 2006 through September 2013. These patients were identified using a variety of testing strategies including single-site testing, multi-site testing (analysis of three AJ founder mutations), or full sequencing and rearrangement testing. We did a matched pair analysis for patients tested during a similar time period who were identified to have a single *BRCA1* or *BRCA2* mutation.

Results: We identified 196 double heterozygous mutation carriers. Of the 196 double heterozygotes, 122 (62.2%) reported a personal history of either breast cancer, ovarian cancer, or both breast and ovarian cancer. The average age of a single breast cancer diagnosis for a double heterozygous mutation carrier was 42.8 years and 51.7 years for a single diagnosis of ovarian cancer.

Conclusions: The average age of cancer diagnoses in double heterozygous mutation carriers was more similar to single *BRCA1* mutation carriers than to single *BRCA2* mutation carriers. Based on our testing population and the increased prevalence of the founder mutations in the AJ population, the majority of the double heterozygous mutation carriers were of Western/Northern European ancestry and AJ ancestry.

P004

Determining the Clinical Significance of Silent *BRCA1* and *BRCA2* Sequencing Variants

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Objectives: Diagnostic sequencing analysis of the *BRCA1* and *BRCA2* genes may identify nucleotide changes that are predicted to be translationally silent. However, additional analyses, such as the use of *in silico* mRNA splice site predictors and direct analysis of patient mRNA, may indicate that some variants cause abnormal mRNA production or splicing and increased risk of breast and ovarian cancer. We describe the algorithm used by our laboratory to determine possible pathogenicity of presumed silent variants.

Methods: Sanger sequencing analysis of *BRCA1* and *BRCA2* identified nucleotide changes predicted to result in translationally silent variants. *In silico* mRNA splice site analysis and scientific literature review identified variants which may result in abnormal mRNA production. Variant pathogenicity was further investigated by history weighting analysis, variant co-segregation with cancer, or biochemical confirmation of an mRNA defect.

Results: Sequencing analysis of >1 million patients identified >2000 unique presumed silent variants in *BRCA1* and *BRCA2*. Splice site analysis identified *BRCA1* c.3699A>G (p.Lys1233Lys) and *BRCA2* c.9876G>A (p.Pro3292Pro) as potentially resulting in abnormal splicing, but our history weighting algorithm strongly indicates these variants to be benign. *BRCA1* c.75C>T (p.Pro25Pro) has been observed in a patient with decreased mRNA transcript levels and was postulated to be pathogenic. However, in our patient population, this variant co-occurs in trans with known deleterious *BRCA1* mutations in 5 patients and has been found in the homozygous state in 13 patients, strongly indicating a benign classification. History weighting analysis confirms the benign nature of this variant.

Conclusions: While the use of *in silico* splice site analyses may predict abnormal splicing, these tools should be used cautiously and predictions rigorously verified by other methods. In addition, analysis of mRNA transcription levels to determine pathogenicity should be used with extreme care as transcript levels may not correlate directly to variant pathogenicity and clinical outcome.

P005

Align-GVGD, SIFT, Polyphen, MAPP-MMR, Grantham Analysis, and Condel Are Weak Predictors of the Clinical Significance for Missense Variants

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Objective: To analyze whether commonly used *in silico* tools, which assess the phylogenetic conservation of a specific amino acid throughout evolution, can accurately characterize the possible disease association of missense mutations, which represent the majority of variants of uncertain clinical significance (VUSs) identified by genetic testing.

Methods: We compared the accuracy of 6 commonly used algorithms (Align-GVGD, SIFT, PolyPhen-2, MAPP-MMR, SIFT, Grantham Analysis, and Condel) using a dataset of 1118 *BRCA1*, *BRCA2*, *MLH1,* and *MSH2* variants previously classified as clinically deleterious or benign by our laboratory's variant classification program.

Results: For all algorithms except Align-GVGD, the false-positive (FP) rate compared to our laboratory's variant classification program was substantially higher than the traditionally accepted threshold for clinical confidence, with a range from 30.6% to 58.5% for *BRCA1*, 27.1% to 40.1% for *BRCA2*, 17.9% to 67.9% for *MLH1,* and 17.1% to 56.1% for *MSH2*. Although the FP rates using Align-GVGD for all 4 genes were lower, including values of 2.2% for *BRCA1* and 7.9% for *BRCA2*, the sample size was too small to provide robust analysis because we excluded 750 variants that were used to train the algorithm. The high FP rates for Condel, which classifies variants based on a weighted average of scores from 5 *in silico* tools, suggests that the use of multiple models is not significantly more accurate than any of the individual models in isolation.

Conclusions: The results of our study suggest that none of the commonly used *in silico* tools achieve the traditionally accepted minimum threshold of specificity for the clinical use of predictive tools.

P006

Changes in *BRCA* Testing and Mutation Spectrum in an Ethnically Diverse Population: An Israeli Perspective

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Mutations in the *BRCA1* and *BRCA2* genes are a major cause of hereditary breast and ovarian cancer in populations worldwide. In many genetic diseases, specific mutations are more common in specific ethnic groups. Thus genetic investigations may begin with targeted mutation testing. In Israel, gene testing by ethnic origin is widely used, particularly in the context of prenatal diagnosis. Benefits are the low cost and known pathogenicity of the mutation. The disadvantages are limited coverage and sensitivity.

BRCA1/2 testing in Israel began shortly after the discovery of 3 prevalent mutations in breast and ovarian cancer patients of Jewish Ashkenazi origins. In the years to follow, founder and prevalent mutations were described in other, but not all, ethnic groups in Israel. All Jewish Diaspora groups are represented in Israel and receive their care through nationwide mandatory health care organizations. In the past few years, the health coverage was expanded to include full gene sequencing. In addition to that, private local facilities offering genetic testing opened, thus reducing prices substantially. These facilitated adequate *BRCA1/2* testing for eligible patients.

In the past 16 years, we identified 250 *BRCA1/2* mutation carriers. In addition to the known ethnic mutations comprising most of our database, we identified mutations in Jews of Syrian, Bukharin, Tunisian, Moroccan, and Sephardic origins. Future work is needed to determine whether these alterations are private or founder mutations. In addition to that, we identified a family of Ashkenazi Jewish ancestry carrying a seemingly private mutation. Surprisingly, we also detected Jewish Ashkenazi mutations in patients of Sephardic, Yemenite, and Persian ancestry. Further insights were gained on patients of Russian non-Jewish backgrounds.

To conclude, we suggest that the combination of mixed populations and availability of cheaper technologies will ultimately waive the ethnic-based testing strategy and lead to performance of whole gene analysis for suspected patients.

BRCA1/2 MUTATIONS, VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE, AND DATABASES (CONTINUED)

P007

Prevalence of *BRCA1* and *BRCA2* Mutations in Unselected Black South African Breast Cancer Patients

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Cancer of the breast is the most common cancer in South African women. Overall, the lifetime risk for developing cancer of the breast is 1 in 29 for all South African women, ranging from 1 in 14 for white women to 1 in 53 for black women (summary statistics of cancer diagnosed histologically in 2004, National Cancer Registry). To date, many studies have been carried out in diverse populations to assess the cancer risks associated with *BRCA1* and *BRCA2* mutations. There is, however, a paucity of data on the role of the *BRCA* genes in women of African ancestry. To determine the proportion of black South African women with breast cancer who carry *BRCA1/2* mutations we screened a hospital-based cohort of 200 patients (unselected for family history of cancer), using PTT and SSCP-Heteroduplex analysis. MLPA analysis was carried out to screen for large rearrangements of *BRCA1*. Mean age at diagnosis of breast cancer was 43 ± 8.2 years, and 11% of patients reported a family history of breast cancer. Information regarding ethnicity was available for 93% of the cohort (53% were Sotho–Tswana, 37% Nguni, and 3% Tsonga speakers). Deleterious mutations of *BRCA1/2* were identified in 7% (14/200) of the women. Four different frame-shift mutations and one novel genomic rearrangement in *BRCA1* were detected in 4.5% of the women. One nonsense mutation and four different frame-shift mutations in *BRCA2* were found in 2.5% of the cohort. *BRCA1* mutation carriers had a mean age of 39.09 ± 8.6 years, and *BRCA2* mutation carriers had a mean age of 39.73 ± 10.2 years at diagnosis. Only 2 of the 14 women with a *BRCA* mutation reported a family history of breast cancer. This assessment of the *BRCA1/2* mutation frequencies in our cohort represent an underestimation, as the screening methods do not efficiently detect all mutations.

P009

BRCA1/2, *CHEK2*, and *TP53* Testing in Czech High-Risk Families with Breast or Ovarian Cancer

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Hereditary cause of breast or ovarian cancer is regularly tested since 1997 at MMCI. So far 3943 probands with breast or ovarian cancer were tested.

Methods: All coding and splice site regions of both genes are screened by HRM [high resolution melting analysis with light scanner (Idaho Tech)] and DHPLC [Wave System 4500 (Transgenomic)]. Sequencing is done on a 3130 Genetic Analyzer (Applied Biosystems). Investigation of large rearrangements of *BRCA1* is done by the MLPA method.

Results: Pathogenic mutation was detected in 928 of 3943 families (23.5%), *BRCA1* mutation in 621 families (97 different mutations), *BRCA2* in 307 families (90 different mutations). Mutations in both the *BRCA1* and *BRCA2* gene were detected in one family only. In 18 families, UV with predicted pathogenic effect was found (class IV–IARC, with probability of pathogenicity 0.95–0.99). In 93 families, other UV variants (class III–IARC, with probability 0.005–0.949) were found. The 10 most frequently found mutations are:

BRCA1: c.5266dupC, c.3700_3704del5, c.181T>G, c.1687C>T, c.213–12A>G, c.68_69del2, del.5–12, del.21–22

BRCA2: c.8537_8538del2, c.7913_7917del5

These mutations represent about 50% of all detected mutations.

The *TP53* gene was tested (sequencing of exons 2–12 and MLPA) in 155 breast cancer patients. In 10 patients (6.5%) mutation was discovered. *CHEK2* gene mutations, c.1100delC, del5567bp, or p.Ile157Thr, were found in only about 3% of cases.

Conclusions: The large spectrum of *BRCA1/2* mutations needs complete gene analysis in all high-risk Czech patients. No hotspot mutations are found. *TP53* and *CHEK2* mutations are infrequently found. Genetic testing is now expanded in families with high-risk family history without detected *BRCA1/2* or *TP53* mutation using TruSight Cancer Target Genes (MiSeq, Illumina) with a panel of 94 genes—tumour suppressor, DNA repair, and proto-oncogenes. The study is ongoing.

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P010

Double Heterozygotes for *PALB2* and *BRCA1*/*BRCA2* Mutation Carriers in French Canadian Hereditary Breast Cancer Patients

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BRCA1 and *BRCA2* mutation carriers are at significantly increased risk for premenopausal breast cancer (BC). While various mutations have been described, specific mutations accounted for ~84% of carrier French Canadian (FC) cancer families due to common founders. Nine specific *BRCA1*/*BRCA2* mutations recur at frequencies such that carrier detection involves a genetic test for these mutations. Other more rare germline pathogenic mutations have been found in *PALB2* and *TP53* in FC cancer families, where *PALB2*:Q775X mutation has been found to recur in this population. When a carrier is identified, a test for the specific mutation identified is then offered to other family members, but rarely includes screening for other mutations. Although double heterozygote (DH) appears to be rare, we have reported previously in our FC cancer families a patient bearing both *BRCA2*:E3002K and *PALB2*:Q775X recurrent mutations.

As mutation analysis in research and clinical setting has usually been limited to screening for the initial mutation, the frequency of the double heterozygotes may be underestimated. This is demonstrated in other ethnically defined populations where mutations recur at high frequencies. We tested for the co-occurrence of a *PALB2*:Q775X and *BRCA1*/*BRCA2* mutation in 230 female breast cancer cases from FC cancer families that harboured a *BRCA1* ($n = 39$) or *BRCA2* ($n = 57$) mutation. Mutation screening was performed using a tailored PCR-based TaqMan mutation screening assay. No additional *PALB2*:Q775X mutation carriers were identified among the *BRCA1*/*BRCA2* mutation carriers. Our results suggest that carriers of *PALB2*:Q775X mutations rarely co-occur in *BRCA1*/*BRCA2* mutation-positive FC breast cancer families.

P011

Prevalence of *BRCA1* and *BRCA2* Mutations in Unselected Breast Cancer Patients from Peru

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Background: Inheritance of a *BRCA1* or *BRCA2* mutation accounts for approximately 5% of all breast cancers. The contribution of *BRCA* mutations to breast cancer in Peru has not yet been explored. It is important that the frequency of mutations be established among unselected cases of breast cancer in Peru to plan genetic and preventive services.

Objective: The aim of this study was to establish the mutation frequencies of the *BRCA* genes in breast cancer patients unselected for family history or age, from Peru.

Methods: We enrolled 300 unselected women with breast cancer from a cancer specialized public hospital in Lima, Peru. A risk factor questionnaire and a saliva sample was obtained from each patient and processed for DNA analysis. Mutations in *BRCA1* and *BRCA2* were sought using a panel of recurrent Hispanic *BRCA* mutations (HISPANEL). All mutations were confirmed by direct sequencing.

Results: Genetic testing was successfully completed for 266 of the 300 cases (89%). Among the 266 cases, 13 deleterious mutations were identified (11 in *BRCA1* and 2 in *BRCA2*), representing 4.9% of the total. The average age of breast cancer in the mutation-positive cases was 44 years. Of the 11 *BRCA1* mutations, 7 were in exon 2 185delAG. Of the 11 *BRCA1* mutations, 4 were in exon 11: 2 in 2080delA, 1 in 943ins10, and 1 in 3878delTA. The *BRCA2* mutation in exon 11 (3036del4) was seen in 2 patients.

Conclusion: The frequency of *BRCA* mutations in unselected breast cancer cases from Peru was found to be approximately 4.9% using HISPANEL. Given the relatively low cost of HISPANEL, it might be time to consider offering this test to Peruvian individuals.

BRCA1/2 MUTATIONS, VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE, AND DATABASES (CONTINUED)

P012

Evaluation of Bahamian *BRCA* Founder Mutations in the Caribbean

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Objectives: The proportion of breast cancer related to mutations in *BRCA1* and *BRCA2* genes varies among populations. In some populations, certain mutations have been reported with high frequency. The islands of the Bahamas have a high prevalence of *BRCA* mutations (27%) in unselected breast cancer cases. Little is known about the prevalence of hereditary breast cancer and *BRCA* founder mutations in other parts of the English-speaking Caribbean. We sought to find out if other islands in the Caribbean harbour a similar prevalence and spectrum of *BRCA* mutations.

Methods: A saliva sample was collected from 347 women with breast cancer in the Cayman Islands, Jamaica, Barbados, Dominica, and Trinidad. A mutation panel was created using 11 Bahamian mutations and 3 additional mutations reported in these islands. We used the Sanger method to sequence 400–600 bp around each of the 14 mutations in the panel.

Results: One panel mutation in *BRCA2* (1538del4) was found in a woman from Barbados. Two non-panel mutations in *BRCA1* (3347delAG, 3484delCA) were found incidentally because they were close enough to the panel mutations to be captured by sequencing of those regions.

Conclusions: The low carrier frequency of the panel mutations and these incidental findings strongly suggest that the mutation spectrum in these Caribbean islands is different from that found in the Bahamas. Whole *BRCA1* and *BRCA2* sequencing should be used for screening patients from these regions.

P013

Comprehensive Functional Analysis of All Documented Missense Variants in the Carboxy Terminal Region of *BRCA1*

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Objectives: *BRCA1* variants of unknown clinical significance impede patients from receiving informative reports from genetic testing for breast and ovarian cancer susceptibility. The *BRCA1* C-terminus (amino acids 1396–1863) contains a coiled-coil motif, a disordered region, and the globular tandem BRCT domain and acts as a transcriptional activator when fused to a heterologous DNA binding domain, which can be assayed to evaluate pathogenicity of individual variants. To date, there are in the population 219 documented unique missense variants in this region of *BRCA1*, yet only 135 (61%) have been functionally investigated using transcriptional assays. This study provides a comprehensive analysis of the transcriptional activation potential of all remaining 84 missense variants.

Methods: Transcriptional activation assays were performed on the set of 84 missense variants in mammalian 293T cells. *BRCA1* constructs are fused to the Gal4 DNA binding domain, which activates transcription of the luciferase reporter. These results were incorporated into a joint analysis of all 219 variants using the VarCall computational model to predict the likelihood of pathogenicity (Iversen *et al. Cancer Epidemiol Biomarkers Prev* 2011;20:1078–88). Further, the likelihood of pathogenicity generated by VarCall for every variant was mapped onto the BRCT domain 3D crystal structure.

Results: This study revealed additional *BRCA1* variants with impaired transcriptional activation and provides the first comprehensive functional analysis of all known variants in this region. Variants that disrupt the coiled-coil motif negatively affect the transcriptional activity of *BRCA1*, though to a lesser extent than other known pathogenic variants. Additionally, 3D mapping of the variants identifies specific secondary structures that are more sensitive to disruption and surface regions likely to contain potential binding sites to other ligands.

Conclusions: Functional analysis of all known missense variants in the C-terminus of *BRCA1* provides a standardized and validated data source to be incorporated into multifactorial statistical models to classify these variants.

P014

High Throughput Genetic Analysis in 68 Hereditary Breast Cancer Patients

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Objective: Here, we report our first results from our HBOC-NGS panel that includes 56 genes associated with breast and ovarian cancer. So far, 68 samples were analyzed in a diagnostic setting.

Methods: A custom NGS panel (HaloPlex, Agilent) was used to target 56 genes. Depending on the patient's family history, a set of "diagnostic" genes (mostly *BRCA1/2*, *RAD51C/D*) as well as "screening" genes were defined. DNA was isolated from all samples, enriched and sequenced on the Illumina MiSeq (2×150 bp paired-end) following standard protocols. For diagnostic genes, regions with low depth (<20×) were complemented by Sanger sequencing as well as MLPA. All mutations were confirmed by conventional Sanger sequencing.

Results: So far, we have sequenced 68 hereditary breast cancer patients. Overall 91%–99% of all targeted exons were represented with a "diagnostic" average depth of >20×. Roughly 70 SNVs were identified per sample, and stringent filtering resulted in less than 7 variants for validation.

We identified several mutations that are known to cause HBOC, as well as mutations likely to cause HBOC. Further experiments and segregation analysis are required to determine the pathogenicity in the latter. In addition, heterozygous mutations were found in genes relevant for different autosomal recessive cancer syndromes.

Conclusion: Taken together, we demonstrate that NGS is a fast and cost-efficient genetic screening tool to analyze for variants in genes associated with the development of hereditary breast cancer. By applying this approach we were able to uncover both known and novel sequence variants.

P015

Non-Founder Mutations in Ashkenazi Jewish Individuals Undergoing Testing for *BRCA1/2*

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Objective: Individuals of Ashkenazi Jewish (AJ) ancestry are at increased risk for carrying a mutation in 1 of 3 specific locations in *BRCA1*/*BRCA2* (187delAG and 5385insC in *BRCA1*, 6174delT in *BRCA2*). These founder mutations account for the vast majority of all deleterious *BRCA* mutations in this ethnic group. There is some debate on whether proceeding to reflex full sequencing and deletion/duplication *BRCA1/2* analysis is warranted in certain AJ families. Due to the significantly increased risk of cancer and strong management implications, it is important to elucidate whether reflex testing provides additional yield in AJ families. This study presents the results of three AJ families identified to carry a non-founder mutation in *BRCA1/2*.

Methods: *BRCA* genetic testing results of AJ individuals who presented for genetic counselling between May 2008 and December 2013 were reviewed. Based on their AJ ancestry, *BRCA1/2* testing was performed in a stepwise fashion, starting with analysis of the three AJ founder mutations. Certain individuals who tested negative for the 3 AJ founder mutations, and who met more stringent family history criteria, proceeded to *BRCA* full sequencing. Excluding their AJ ancestry, each of these families still met National Comprehensive Cancer Network (NCCN) guidelines for *BRCA1/2* genetic testing.

Results: Three AJ families tested were found to carry a deleterious mutation outside the AJ founder sites. Two tested positive for a *BRCA2* mutation, (4075delGT and 3036del4) and one tested positive for a *BRCA1* mutation (4035delTT). Each family had a significant cancer family history, including early onset breast, male breast cancer, and/or ovarian cancer.

Conclusion: This series demonstrates the occurrence of non–AJ founder mutations in a minority of patients with significant family histories undergoing testing for *BRCA*. These results may have implications for families who, despite having a strong family history, test negative for the 3 AJ founder mutations.

BRCA1/2 MUTATIONS, VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE, AND DATABASES (CONTINUED)

P016

Development of a Next-Generation Sequencing Panel for Hereditary Breast and Ovarian Cancer

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Objectives: The aim of this study was the development and evaluation of a next-generation-sequencing (NGS) panel for HBOC. We designed a 56-gene panel, analyzed 20 control samples, and validated the results with Sanger sequencing.

Methods: 56 Genes known to be associated with breast and ovarian cancer were selected for a custom enrichment sequencing panel (HaloPlex, Agilent). 20 Samples from HBOC patients were used as controls and sequenced on the Illumina MiSeq system (paired end, 150 bp/read) following the manufacturer's protocols.

Results: An average of 2.9×10^6 reads ($\pm8.4\times10^5$ reads) was generated and a read depth of 1170× (±354×) on target was obtained. Of the target region, 97.3% was at least covered with 20× (±2.12%). We detected an average of 89 variants (±9 variants) per patient and panel. All sequence variants that were found in *BRCA1/2* were confirmed by Sanger sequencing ($n = 143$, 141 SNVs and 2 small indels). It is worth mentioning that 2 detected SNVs were located within a homopolymer stretch with a length of 7 nucleotides. All pathogenic mutations within our positive controls were confirmed by NGS. We are currently working on detection of large deletions/insertion (for example, whole exons) and closing coverage gaps within the target region (for example, in *BRCA2*). Especially the latter will reduce time and effort required for complementary experiments.

Conclusions: NGS seems to be a valuable diagnostic tool in HBOC. We were able to validate all variants found by NGS with Sanger sequencing and, even more important, all pathogenic mutations within our positive controls. Further steps ahead are optimization of our sequencing panel and detection of large indels.

P017

Development, Validation, and Early Experience with a Next-Generation Sequencing Assay for *BRCA1* and *BRCA2* Mutations

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Objectives: We developed a *BRCA* next-generation sequencing (NGS) assay using bait tile library exon capture. This report describes the development and validation of the assay and experience with the initial 521 subjects tested using 2 different NGS instruments.

Methods: For validation, we used DNA from 27 subjects with 27 different known deleterious mutations in either *BRCA1* or *BRCA2,* and 67 consented adult volunteers with 67 different benign polymorphisms. We analyzed DNA samples with both the Illumina MiSeq platform (MiSeq Reporter software V2.3) and the Life Technologies Personal Gene Machine [PGM (Torrent Suite V.3.6.2)]. The vendor-supplied MiSeq Reporter software repeatedly did not detect 2 known deleterious *BRCA1* mutations (c.1175_1214del40 and c.3481_3491delGAAGATACTAG). However, we developed a proprietary alignment algorithm (QSAP) that detects both mutations using the MiSeq instrument.

Results: The MiSeq (with QSAP) and PGM platforms consistently detected all 27 known mutations, including the 2 deletions. Thus, the platforms each achieved 100% sensitivity. Specificity was 100% for the MiSeq and approximately 96% for PGM. We began clinical testing using both platforms for all patients. Discrepancies were resolved by manual data review. Analysis of the initial 521 reported results revealed 35 variant call discrepancies between MiSeq and PGM in >2000 variant calls. Discrepancies were resolved by manual review of the data or repeat analysis. The single MiSeq error (missed benign polymorphism) was due to a combination of low coverage and strand bias. Adjusting the filtering parameters corrected this error and allowed identification of the variant. All other errors were in PGM analysis, the most consequential being 2 false-negative results for pathogenic mutations: a 60-bp deletion and a 10-bp insertion in *BRCA1*.

Conclusions: The MiSeq instrument coupled with our proprietary QSAP alignment algorithm is a robust platform capable of highly accurate *BRCA* sequence analysis.

P018

Enhancement of VUS Interpretation by Multiple Algorithm in Familial Breast Cancer

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Objectives: We have previously sequenced *BRCA1*, *BRCA2*, *TP53,* and *PTEN* in the peripheral blood samples of 464 patients and 100 local healthy individuals using 454 GS Junior System. Approximately 12.5% of patients (58 of 464) were found to carry a total of 60 missense variants of unknown significance (VUSS) which could not be found in the 1000 Genomes Database. In this study, we aimed to develop a multifactorial algorithm which includes family data to assess the clinical significance of these VUSS.

Methods: Several approaches have been used to classify these 60 missense VUSS which include 1) evaluation of the VUS frequencies in 100 normal controls; 2) co-occurrence of VUS in trans phase with known deleterious mutations; 3) indications from multiple *in silico* algorithms: PolyPhen, SIFT, and Align-GVGD; 4) odds in favour of causality of the VUSS; 5) co-segregation of VUSS with disease in families; and 6) clinicopathologic data for these VUS carriers.

Results: 1 *BRCA1* and 3 *BRCA2* VUSS were observed in unaffected individuals, while 1 *BRCA2* VUS was found to co-occur in trans phase with known deleterious mutant, suggesting neutrality of these VUSS. After initial classification of the 60 missense VUSS by multiple *in silico* analyses, 21 VUSS were assigned to class III (likely pathogenic), and 39 VUSS were assigned to class II (likely benign). Of 6 VUSS where family data were available, 3 class II and 1 class III were re-classified to class I (benign), 1 class II was re-assigned to class III, and 1 class III VUS remained unchanged.

Conclusions: Clinicopathologic data showed that patients with family history were associated with an increased risk of *BRCA*-related deleterious mutation ($p < 0.0001$), but not associated with that of VUS, suggesting that a significant number of VUSS were benign. Among 60 missense VUSS, 9 showed evidence of neutrality, and 1 showed elevation in pathogenicity. A multifactorial algorithm including family data is important during VUS assessment as *in silico* analysis alone is not always sufficient for the interpretation of a variant.

P019

The Contribution of *BRCA1* and *BRCA2* Mutations to Ovarian Cancer in the Lviv Region, Ukraine

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Ovarian cancer is the fifth most common cancer among women in Ukraine. There are 4000 new cases annually (compared to 2600 in Canada). Mutations in *BRCA1* and *BRCA2* account for a high proportion of all cases in Slavic countries of Eastern Europe (13% of ovarian cancer cases in Poland, 16% in Belarus, 19% in Russia). The contribution of *BRCA1* and *BRCA2* mutations to ovarian cancer in Ukraine has not been studied and genetic counselling and testing for cancer mutations are in their infancy. Founder mutations are common in Poland and other countries, and as a consequence, genetic testing can be done inexpensively. Ukraine is bordered by Poland, Belarus, Russia, Moldova, Romania (non-Slavic), Hungary (non-Slavic), and Slovakia. We predict that the prevalence of *BRCA1* and *BRCA2* mutations in Ukraine might be similar to or even higher than in those countries. We sought the presence of 13 mutations previous identified in Slavic populations by previous studies among 100 unselected invasive epithelial ovarian cancer patients. Eight *BRCA1* mutations (5370C/T, 300T/G, 4153delA, 794delT, 185dAG, 3819del5, 5382insC, 3875del4) and five *BRCA2* mutations (886de1GT, 4075de1GT, 5467insT, 6174de1T, 8138de15) were selected for study. A substantial number of hereditary ovarian and breast cancers might be prevented in Ukraine by identifying women at risk of carrying *BRCA1* or *BRCA2* mutations and by offering them genetic testing and appropriate prophylactic measures.

CLINICAL ISSUES IN CANCER SURVEILLANCE AND MANAGEMENT

P020

Timing of Oral Contraceptive Use and the Risk of Breast Cancer in *BRCA1* Mutation Carriers

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Objectives: It is not clear whether early oral contraceptive use increases the risk of breast cancer among young women with a *BRCA1* mutation. Given the importance of oral contraceptive use for the prevention of ovarian cancer, estimating age-specific risk ratios for oral contraceptive use and breast cancer is important.

Methods: We conducted a case–control study of 2373 matched pairs of *BRCA1* mutation carriers. Breast cancer cases and unaffected controls were matched on year of birth and country of residence. Detailed information about oral contraceptive use was collected from a routinely administered questionnaire. Conditional logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for the association between oral contraceptive and breast cancer, by age at first use and by age at diagnosis.

Results: Oral contraceptive use was significantly associated with an increased risk of breast cancer for women who started the pill prior to age 20 (OR: 1.35; 95% CI: 1.11 to 1.46; $p = 0.002$). The effect was limited to breast cancers diagnosed before age 40 (OR: 1.28; 95% CI: 1.05 to 1.57; $p = 0.02$); the risk of early-onset breast cancer increased by 10% with each additional year of pill use when initiated prior to age 20 (OR: 1.10; 95% CI: 1.02 to 1.19; $p = 0.02$). There was no observed significant increase in risk for women who started using the pill after age 20 (OR: 1.08; 95% CI: 0.92 to 1.28; $p = 0.34$) or for women diagnosed at or after the age of 40 (OR: 0.99; 95% CI: 0.80 to 1.22; $p = 0.93$).

Conclusions: Oral contraceptive use before age 20 increases the risk of early-onset breast cancer among women with a *BRCA1* mutation, and the risk increases with duration of use. Caution should be taken when advising women with a *BRCA1* mutation to take oral contraceptives prior to age 20.

P021

Use of Hormone Replacement Therapy After Risk-Reducing Salpingo-oophorectomy

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Objectives: Risk-reducing salpingo-oophorectomy (RRSO) gives the most effective prevention of ovarian cancer in women with *BRCA* mutations. Among premenopausal women, RRSO leads to abrupt surgical menopause and may cause several unwanted health effects. After bilateral oophorectomy at young age, and without replacement of hormones, women may have increased mortality, increased cardiovascular risk, reduction of cognitive function, increased bone loss, and increased postmenopausal complaints. Norwegian guidelines recommend prescription of systemic hormone replacement therapy (HRT) immediately after RRSO to premenopausal women with no history of breast cancer. We aimed to investigate the use of HRT after RRSO.

Methods: Retrospective cohort study identified 503 women who had undergone RRSO between 1990 and 2005 through surgical records from 3 Norwegian university hospitals. Of these, 324 gave written informed consent and provided all necessary information. The women completed a questionnaire about demographic conditions and general health, and a specific questionnaire about use of HRT. Our primary outcome was the proportion of the women who were current users of systemic HRT.

Results: Median age at survey was 54 years, and median age at RRSO was 48 years. Altogether, 98 women (30%) used systemic HRT. In the age group <45 years, 67% used systemic hormones. Among women <53 years (median age of natural menopause in Norway) and without a history of breast cancer, 55% used systemic hormones. A history of no breast cancer [odds ratio (OR): 8.49; 95% confidence interval (CI): 3.29 to 21.9] and higher level of education (OR: 2.45; 95% CI: 1.36 to 4.41) was associated with HRT use in multivariable logistic regression analysis.

Conclusion: Fewer women than expected used systemic HRT after RRSO. Remarkably few hormone-eligible women were present users. Improved guidelines for hormone supplementation after RRSO are needed to optimize quality of life and lower risk of long-term morbidity.

P024

Development of a Risk Assessment Tool for Targeted Population-Based Mammographic Screening

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Population-based mammographic screening is the main form of detection of breast tumours at a stage where they are more amenable to successful treatment. However women are offered the same age-based screening regime even though it is known that they can differ substantially in their risk of developing breast cancer. We hypothesize that incorporating genetic factors using a polygenic risk score (PRS) and non-genetic risk factors, including mammographic density, will improve detection rates. As a first step in building the resources to improve mammographic screening, we have established LifePool (http://www.lifepool.org), which is a prospective cohort of >75,000 healthy women attending BreastScreen Victoria, Australia. LifePool participants give informed consent to access to all screening and cancer incidence data and complete a health and lifestyle questionnaire. More than 50% of women consent to provide DNA for use in germline genetic research.

Using this cohort, we will identify the optimal weighted combination of PRS, breast density, and other non-genetic risk factors to predict observed screen-detected cancer (SDC) and interval cancer (IC) rates in the cohort. Using these data we will specify models of personalized screening strategies and identify which optimize SDC rates and reduce IC rates while containing or reducing false-positive outcomes.

Simulations of a hypothetical cohort of 20,000 women provided data highly suggestive of a large benefit associated with inclusion of a PRS to screening schedules. For example, making screening annual in the high-risk group could reduce the interval cancer rate by more than half in the top PRS quartile group. While these predictions are intriguing, they are based on a number of assumptions that are not necessarily reflective of a real life screen population. Our prospective study will provide robust data from a carefully controlled and representative screened population that can be used to progress to tailored screening trial.

P025

Development of an S-G2 Micronucleus Assay for the Detection of *In Vitro* Chromosomal Radiosensitivity in *BRCA1* and *BRCA2* Mutation Carriers

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BRCA1 and *BRCA2* play an important role in the DNA damage response pathway. Mutations in these genes may be associated with increased radiosensitivity, an important issue as several guidelines advise regular mammography screening from young age in *BRCA1* and *BRCA2* mutation carriers.

To determine the degree of radiosensitivity in *BRCA1/2* mutation carriers, different assays have been developed, which however failed to provide consistent results. For instance, the classic G0 micronucleus assay, based on the irradiation of quiescent lymphocytes (G0 phase of the cell cycle), is not suitable to unequivocally determine radiosensitivity in *BRCA1/2* mutation carriers. In G0, only nonhomologous end joining is available for the repair of radiation-induced double-strand breaks (DSBs). *BRCA1/2*, however, are mainly involved in the homologous recombination (HR) pathway for repair of DSBs and/or in G2/M checkpoint control. These two pathways are active only within the late S and G2 phase of the cell cycle.

In this study we optimized an S-G2 micronucleus assay in lymphocytes allowing detection of defects in HR and G2/M cell cycle control. Results obtained with this assay in a pilot study showed significantly increased radiation-induced micronucleus yields in 10 *BRCA1* mutation carriers compared to healthy controls. Results were not statistically significant for 10 *BRCA2* mutation carriers.

We are currently extending this study to a larger group of *BRCA1/2* mutation carriers. Our results may help to establish whether benefits from early mammographic screening in asymptomatic mutation carriers outweigh the risks linked to increased cumulative doses of radiation. Furthermore, our test may have the potential to be used as a universally applicable functional test for analysis of variants of unknown clinical significance, at least in the *BRCA1* gene, without the need of cloning and transfection of these variants in cell lines.

CLINICAL ISSUES IN CANCER SURVEILLANCE AND MANAGEMENT (CONTINUED)

P026

Perceived Benefits of and Barriers to Risk Management Among Unaffected *BRCA* Mutation Carriers

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Objectives: Prophylactic surgery and surveillance can reduce cancer morbidity and mortality in *BRCA* mutation carriers, but only if carriers adhere to recommended cancer risk management. Determining carriers' perceived benefits of and barriers to risk management behaviors can inform interventions to improve adherence. But, there are few such data for the full range of risk management behaviors. As part of a study of adherence to current risk management guidelines, we asked participants to list perceived benefits and barriers to surveillance and prophylactic surgery.

Methods: A telephone survey was conducted among unaffected female *BRCA* mutation carriers age 25+ from Duke and UNC hereditary cancer clinics. Perceived benefits of and barriers to each of 6 behaviors (mastectomy, oophorectomy, mammography, breast MRI, CA125 testing, and pelvic ultrasonography) were assessed via an open-ended items. Responses were content-analyzed. The proportion of participants listing each benefit and barrier was calculated as a frequency effect size.

Results: Study sample ($n = 97$) was primarily Caucasian, employed, college graduates and privately insured. For all behaviors, risk reduction was the most commonly mentioned perceived benefit. Lists of perceived benefits for surveillance behaviors were short, save for breast MRI. Lists of perceived barriers were longer for all behaviors, though most barriers were mentioned by <5% of participants. The exception was mastectomy, with at least 6% of participants listing 13 barriers, including breastfeeding considerations (16%), surgical complications (11%), body image (9%), incomplete risk reduction (7%), and emotional ramifications (6%).

Conclusions: Given high agreement among unaffected *BRCA* mutation carriers on the perceived benefits of risk management, interventions to improve adherence to recommended management should focus on addressing perceived barriers. The long and individualized list of perceived barriers mentioned in our study indicates that an intervention tailored to address barriers must be flexible enough to anticipate and respond to each, particularly those for prophylactic mastectomy.

P028

Cardiovascular Health, Bone Density, and Quality of Life after Risk-Reducing Salpingo-oophorectomy: The PROSper Study

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Objectives: Current guidelines recommend risk-reducing salpingo-oophorectomy (RRSO) for women with a *BRCA1* or *BRCA2* mutation by age 40 to reduce the risk of hereditary ovarian, tubal, and breast cancer. RRSO in this age range results in a premature menopause that may have adverse consequences on cardiovascular health, bone density, and quality of life. The goal of our study is to identify long-term health consequences of RRSO to inform patient decision-making and guide clinical management to optimize long-term survival and well-being.

Methods: The PROSper (Prospective Research on Salpingo-oophorectomy) Study is a prospective cohort study of 100 women age 35–50 years with a *BRCA1* or *BRCA2* mutation who elect to undergo either RRSO with a fine sectioning protocol or nonsurgical management. Over 3 years of follow-up, changes in cardiovascular health, bone health, and quality of life will be assessed. Cardiovascular risk is evaluated with carotid artery intima media thickness (IMT), an independent predictor of myocardial infarction and stroke. Dual-energy X-ray absorptiometry (DXA) scans are used to evaluate fracture risk. Quality of life is assessed with standardized questionnaires. Multivariable regression models will be used to evaluate differences in the change of these outcomes between women who undergo RRSO and age-matched controls who do not undergo surgery.

Results: Since recruitment began in October 2013, 20 women have enrolled in the study: 7 in the RRSO group, and 13 with ovarian preservation. Of women in the RRSO group, 70% are 35–40 years old. Of participants, 50% have a history of breast cancer and 35% have a *BRCA2* mutation. Of women invited to participate in PROSper, 85% have enrolled.

Conclusions: The PROSper study will fill an important gap in our knowledge of RRSO management by identifying risks of noncancerous diseases following RRSO that decrease long-term survival and impair quality of life.

P029

The Effect of Coffee Consumption on the Total Antioxidant Potential in Women With and Without a *BRCA1* Mutation

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Objectives: Caffeinated coffee consumption has been associated with a significant reduction in breast cancer risk among women with a *BRCA1* mutation. Given that the Brca1 protein has been implicated in the antioxidant response pathway, higher levels of oxidative stress may play a role in cancer development among mutation carriers. Coffee contains several active ingredients with antioxidant properties. The objective of this study was to assess whether coffee consumption is associated with lipid peroxidation and protein oxidation in women with and without a *BRCA1* mutation.

Methods: The current study included 25 *BRCA1* mutation carriers and 25 non-carrier controls. Daily total coffee, caffeinated coffee, and decaffeinated coffee consumption (cups/day) was collected using an established research questionnaire. Lipid peroxidation (serum MDA per µmol/L) was measured using the thiobarbituric acid–malondialdehyde (TBA-MDA) assay, and protein oxidation (serum thiols per µmol/L) was measured by the loss of reduced thiol (–SH) groups using 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) assay. The relationship between coffee consumption and oxidative stress was evaluated using multiple regression analysis.

Results: There was no significant association between increasing total coffee consumption (women who consumed more than 3 cups of coffee/day versus women who consumed 3 cups of coffee or less/day) and levels of lipid peroxidation (10.36 µmol/L vs. 10.07 µmol/L, $p = 0.22$) or protein oxidation (382.07 µmol/L vs. 397.16 µmol/L, $p = 0.08$). Stratification by *BRCA1* mutation status and by decaffeinated and caffeinated coffee also showed no significant association.

Conclusion: Increasing coffee consumption was not associated with levels of oxidative stress as measured by lipid peroxidation and protein oxidation in this group of women. The effect of coffee consumption on DNA oxidation as a marker of oxidative stress needs to be examined. Given the role of the Brca1 protein in the antioxidant response pathway and the known chemopreventive properties of coffee, the role of coffee needs to be studied further in a larger population of *BRCA1* carriers.

P030

Qatar Experience of the Breast/Ovarian Cancer High-Risk Clinic

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Background: Breast cancer is the most common cancer in Qatar. The incidence rates increased from 45 per 100,000 in 2003–2007 to 56 per 100,000 in 2008–2011. Qatari patients accounted for 32% of all the diagnosed breast cancers in females. The prevalent age group was 40–50 years old (36% of all affected women).

Objective: To describe Qatar experience of the breast/ovarian cancer high-risk clinic (BOHRC) for incidence of HBOS and management of individuals within high-risk categories, and to find out if there are any particular mutations related to *BRCA1* and *BRCA2* genes.

Methods: Data were collected from 20 March to December 2013. Based on our guidelines, eligible women undergo detailed assessment, including lifetime risk for developing breast cancer and genetic scoring. Using international scoring tools, patients who score ≥10% for *BRCA1/2* mutations would be offered genetic testing or surveillance if lifetime risk is ≥25%. All cases are discussed in the multidisciplinary meeting to plan surveillance and risk reduction strategies.

Results: 143 cases were seen in the BOHRC, 78 patients were assessed by the genetic counsellor, and 42 high-risk patients were offered genetic testing. Of those tested, 21 were *BRCA1*-positive, 1 was *BRCA2*-positive, 6 had variants of uncertain significance, 2 had three variants of uncertain significance, and 12 were *BRCA1/2*-negative. Remaining patients were either not eligible for genetic testing but with an increased lifetime risk, or at low risk with standard lifetime risk, or were at high risk but refused genetic testing. Detailed report of the mutations will be submitted in actual abstract.

Conclusion: The incidence of *BRCA* mutations does exist in our population and contributes to the high incidence of breast cancer in younger age group. Our findings are worth further research.

CLINICAL ISSUES IN CANCER SURVEILLANCE AND MANAGEMENT (CONTINUED)

P031

Risk-Reducing Surgery for Hereditary Breast and Ovarian Cancer Syndrome: A Single-Institution Experience in Japan

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Objectives: Salpingo-oophorectomy (RRSO) and mastectomy (RRM) are standard management procedures for *BRCA* mutation carriers. In Japan, most institutions do not offer RRSO and/or RRM. Our hospital began *BRCA* genetic testing, followed by RRSO and/or RRM early. We report the current status of RRSO and/or RRM at our hospital.

Methods: Clinical charts were reviewed between December 2006 and October 2013. The number of RRSO and/or RRM and the relation with breast cancer surgery was assessed.

Results: A total of 220 women underwent *BRCA1/2* genetic testing, which revealed 44 women (20%) with *BRCA1/2* mutations; 17 among them (38.6%) underwent RRSO and/or RRM. Among the 17 women, 11 (64.7%) underwent RRSO and/or RRM with simultaneous breast cancer surgery. Among 6 women who underwent RRSO and/or RRM after breast cancer surgery, 5 (83.3%) were introduced from other hospitals. One woman with triple-negative breast cancer underwent neoadjuvant chemotherapy followed by partial mastectomy with whole breast irradiation at another hospital. After 1 year, she was shown to have a *BRCA1* variant of uncertain significance. As she strongly hoped that she received RRSO and bilateral RRM, she was referred to our hospital. We used PET-CT for the detection of distant metastasis before surgery, which revealed regional and distant nodal metastases. Therefore, she did not undergo RRSO and RRM.

Conclusions: All women who underwent RRSO and/or RRM were previously diagnosed with breast cancer. Most women at our hospital underwent risk-reducing surgery along with breast cancer surgery. Conversely, women who were recommended from other hospitals to our institution for RRSO and/or RRM had already undergone breast cancer surgery. We should discuss the risk of breast cancer recurrence and recommend imaging tests for the presence of contralateral breast cancer and distant metastasis to utilize the advantages provided by RRSO and/or RRM.

P032

Profile of *BRCA* Testing in Ovarian Cancer Patients in the United States and European Union

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Objectives: About 13% of patients with ovarian cancer harbour mutations in the *BRCA* genes. Their disease has distinct characteristics which may impact treatment pathways. However, currently only a selected subset of ovarian cancer patients are offered testing for *BRCA* mutation, based largely upon family history of related tumours. Rates of, and barriers to testing are not well characterized; therefore, our objective was to describe the current *BRCA* testing practices among women diagnosed with ovarian cancer.

Methods: A survey of 465 oncologists, 513 pathologists, and 97 laboratory directors, including detailed questionnaires for 435 ovarian cancer patients, across the United States, United Kingdom, France, Germany, Italy, and Spain was conducted during 2013 by Ipsos Healthcare syndicated MDx Monitor.

Results: Physician awareness of *BRCA* testing in ovarian cancer is strong: 61%–86% in the European Union and more than 90% in the United States. 50%–67% of oncologists in the European Union, compared with more than 80% in the United States, claim to have used *BRCA* testing within 6 months. On average, between 24% (United States) and 7% (United Kingdom) of ovarian cancer patients were tested for *BRCA* post-diagnosis, with large variations between physicians. Guidelines and patient requests were drivers for testing; barriers included perceived lack of clinical utility/targeted therapies (~35%), lack of *BRCA* risk factors (~5%–70% depending on country), patient refusal (~5%), and reimbursement/cost (~15%). In all markets, both germline and somatic *BRCA* testing were common. Testing was generally performed in remote laboratories for efficiency. In the United States, most testing was conducted by Myriad; in European Union, several different tests, including 5% of locally developed tests, were used.

Conclusions: While there is broad knowledge and use of *BRCA* testing, the survey highlighted significant barriers which may prevent ovarian cancer patients from being offered the test. Ongoing research aims to demonstrate the value of testing a broader cohort of patients who may benefit from more personalized treatment approaches.

P033

Do *BRCA1/2* Non-carriers Contribute to Their Doctors' Awareness of Their Genetic Status and Does This Influence Their Own Follow-Up?

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Background: There are reports that cancer screening practices of female non-carriers from *BRCA1/2* mutation–positive families often exceed those recommended for women in the general population, but the reasons for such "overscreening" are unknown. Because most of these women are referred to their family physicians and gynecologists for future risk management, one hypothesis is that risk information based on the genetic test may not be properly transmitted to the usual care settings.

Objective: To explore how family physicians and gynecologists are informed of the mutation status of their true negative patients and if the way this information is conveyed influences their risk management decisions.

Methods: Data about breast and ovarian cancer screening were collected from 216 unaffected female non-carriers from *BRCA1/2* mutation–positive families from 4 cancer genetics clinics in Montreal and Quebec City (mean age: 50 years; mean time since result disclosure: 4.6 years).

Results: Most participants were regularly followed by a family physician (n = 197) or a gynecologist (n = 101). 105 Participants (49%) reported having received cancer screening recommendations at the time of result disclosure, but only 36 (17%) in written form. Although 41 non-carriers (19%) mentioned that a copy of these recommendations was sent to their family physician or gynecologist, 84% declared that their doctors were aware of their genetic status. 117 Participants agreed with their doctor about the frequency in which they should have screening mammograms, and 10 asked to have more frequent screening mammograms than advised by their doctor.

Conclusion: Taken together, our results suggest that many true non-carriers themselves inform their doctor of their test result and that some of them take part in their risk management follow-up decisions. Improving the communication between cancer genetics clinics and usual care settings has the potential to optimize the follow-up of female non-carriers from *BRCA1/2* mutation–positive families.

P034

Assessing the Impact of *BRCA1* Predictive Testing on Breast Cancer Characteristics and Treatment in Northern Ireland

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Objectives: Predictive testing for germline *BRCA1* mutations in unaffected individuals may allow risk-reducing intervention. This study assessed whether the introduction of predictive testing in 1996 altered the characteristics of *BRCA1*-mutant breast cancers presenting in Northern Ireland (NI).

Methods: Cases of *BRCA1* mutated breast cancer were identified from the regional Genetics Department database and the NI Clinical Oncology Information System, and further data obtained from clinical records. Patients were divided into four cohorts: pre-testing, first 5 years post-testing, second 5 years post-testing, and beyond 10 years.

Results: From 1996 to 2013, 72 women with a *BRCA1* mutation and breast cancer had adequate information for inclusion. There was no difference in the average age of presentation between the groups; however, there was a marked reduction in the time interval between cancer diagnosis and postoperative *BRCA1* mutation testing across the groups from 11.25 years (in the earliest group) to 1.22 years (in the latest group).

There was no difference in stage, grade, or histologic type between the groups. There was a trend towards smaller tumours in the later cohorts (4.9 cm to 2.56 cm). Paradigm changes in breast surgery were reflected in these cohorts; there was increased use of sentinel lymph node biopsy and breast reconstruction (6.3% to 40.9%) in later groups. Later tumours were more likely to be classified as triple-negative due to increased estrogen receptor, progesterone receptor, and HER2 testing.

There were no significant differences between use of chemotherapy, radiotherapy or hormone therapy. Patients in the later cohorts were more likely to have further risk-reducing breast and/or ovarian surgery.

Conclusion: This study shows that the introduction of routine testing for *BRCA1* mutations has not significantly changed the characteristics of cancers detected in these patients. However, patients are now more likely to have reduced axillary surgery, to undergo reconstruction, and to have additional risk-reducing surgery.

CLINICAL ISSUES IN CANCER SURVEILLANCE AND MANAGEMENT (CONTINUED)

P035

No Change in the Rate of Bilateral Mammographies After *BRCA1/2* Testing Among True Non-carriers

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Introduction: The majority of women who are non-carriers of the *BRCA1/2* familial mutation may be reassured that they are no longer considered at high risk for breast and ovarian cancer. For this reason, most should be encouraged to adopt the same cancer screening practices as those recommended to women of the same age in the general population.

Objective: The aim of this study is to compare the rate of bilateral mammographies after *BRCA1/2* testing to that prior.

Methods: Information from the Quebec Health Insurance Board (RAMQ) was used to identify all registered bilateral mammographies done between May 1, 1998, and March 31, 2012, in a cohort of 143 French Canadian unaffected true non-carriers. The Cox proportional hazards model for repeated events, with women's age as the time scale, was used to obtain hazard ratios of bilateral mammographies.

Results: The rate of mammographies did not change after *BRCA1/2* testing, neither globally (HR: 0.93; $p = 0.22$), nor by age (<50 years HR: 0.81;, $p = 0.13$; >50 years HR: 1.01; $p = 0.84$). Although women <50 years had a lower rate of mammographies than women >50 years (HR: 0.55; 95% CI: 0.43 to 0.70) after genetic testing, 74% still continued to be screened, which is not generally recommended to women of the same age group in the general population.

Conclusion: In our cohort of true non-carriers of familial of *BRCA1/2* mutation, genetic testing information did not have a significant effect on mammography screening of true non-carriers. Clear-cut recommendations for the follow-up of non-carriers of the *BRCA1/2* familial mutation are needed.

P036

A Study of Bone–Mineral Density in Postmenopausal Women With/Without *BRCA* Mutations: Demographics from the NYU Lynne Cohen Foundation Registry

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Transgenic mice with tissue-specific *BRCA1* deletion exceed the height and bone density of wild-type animals (data from Louis Dubeau's laboratory at the University of Southern California). Differences in women's height linked to *BRCA* mutation–related genetic background have emerged from large databases, but details on specific populations and bone–mineral density (BMD) changes are lacking. Moreover, clinical data must account for multiple factors affecting BMD. The current retrospective chart review at New York University analyzes BMD by dual-energy X-ray absorptiometry (DEXA) in women with known *BRCA*-carrier status vs. those that tested negative for *BRCA* mutations. Entry criteria required registry women to be postmenopausal (spontaneous or surgical) and at least 1 year free of any cancer diagnosis, and completion of cancer treatments. Age, ethnicity, comorbidities, and intake of aromatase inhibitors (AIs), raloxifene, or tamoxifen for prevention, or other medications, including steroid hormones by any route, were recorded. Median age of 29 *BRCA* mutation carriers was 53 years (range: 29–70 years) and of 54 non-carriers was 60 years (range: 37–79 years). Ethnicity was predominantly Caucasian with 31% of carriers and 38% of control subjects reporting Jewish origin. In both groups, relevant comorbidities (such as arthritis, asthma) were found in <10%; at least 20% were on agents to improve "bone health," reflecting a health-conscious population.

Conclusion: The Lynne Cohen Foundation Registry is suitable for future BMD analysis by adding more subjects and including other registry participants (University of Southern California and University of Alabama).

P037

A Clinic-Based Population of *BRCA1* and *BRCA2* Mutation Carriers Diagnosed with Breast Cancer Under Age 30: Can the Family History Predict the Optimal Age to Start Screening?

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Objectives: Clinical guidelines for the management of hereditary breast and ovarian cancer syndrome (HBOC) associated with *BRCA1* or *BRCA2* mutations exist, with variability in the optimal age to begin breast cancer surveillance for unaffected carriers. The HBOC guidelines from the National Comprehensive Cancer Network (NCCN) indicate that breast screening should begin at age 25. In Ontario, through the Ontario Breast Screening Program (OBSP), women with HBOC are eligible to begin breast screening at age 30. Clinical judgement may be used to recommend screening under age 30; a common strategy is to start breast screening 10 years earlier than the youngest age of onset in the family. We reviewed our clinical database to determine if family history appropriately predicts the optimal age to start screening.

Methods: Our clinic database includes 441 known female *BRCA1* or *BRCA2* carriers. We reviewed known carriers within these families with a diagnosis of breast cancer ≤30 years old. A 3-generation pedigree was reviewed to ascertain the youngest age of onset beside the proband.

Results: Six *BRCA1* and eight *BRCA2* mutation carriers were identified with breast cancer diagnosed ≤30 years old (range: 24–30 years; mean: 28 years). Of these women, 42.8% (6/14) had a family history of breast cancer under the age of 40. Therefore, 57.2% (8/14) of these *BRCA1* and *BRCA2* carriers would likely not have been offered screening until age 30 had their mutation status been known. Following NCCN guidelines, 92.8% (13/14) would have been offered surveillance prior to their diagnosis.

Conclusions: Breast cancer ≤30 years remains rare in a clinic-based population of *BRCA1* and *BRCA2* carriers, but cannot consistently be predicted by the age of breast cancer diagnoses in the family. To ensure equitable access to high-risk screening, standardized national guidelines for women at high risk to develop breast cancer need to be implemented across Canada.

P038

BRCA Genetic Testing in the Underserved Medical Setting: Preliminary Findings and Outcomes

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Objectives: 1. Describe results of *BRCA* testing in underserved ethnic minority breast/ovarian cancer patients. 2. Assess risk-reducing surgery uptake in *BRCA*-positive women in this cohort.

Despite guidelines supporting cancer genetic testing, integration of services has lagged in underserved settings. To address this need, USC Norris Comprehensive Cancer Center provided funding to establish services at Los Angeles County and University of Southern California (LAC+USC) Medical Center.

Methods: An IRB-approved retrospective chart review was conducted of breast and/or ovarian cancer patients undergoing genetic counselling at LAC+USC from January 2008 to January 2013. Surgical history was extracted for *BRCA* mutation carriers, excluding those under the recommended age of prophylaxis, undergoing active therapy, or with metastatic cancer.

Results: Genetic counselling was performed in 550 patients: 446 (81%) Hispanic, 49 (8.9%) Asian, and 29 (5.3%) African American. *BRCA* testing was offered to 512 individuals, and 5.4% declined. The 80 identified *BRCA* mutation carriers equalled a 16.91% positivity rate. The majority of the patients tested were women with 1 breast cancer ($n = 472$); however, 42 women with bilateral cancers, 29 ovarian cancer patients, 7 patients with breast and ovarian cancer, and 4 men with breast cancer were included.

Of affected mutation carriers, 42 were offered risk-reducing surgical management, bilateral salpingo-oophorectomy (RRSO), and/or mastectomy (RRM). Of 32 who underwent risk-reducing surgery, 11 received RRM plus RRSO; 10, RRSO only; and 11, RRM only. Presence of an affected family member diagnosed at <35 correlated with uptake of risk-reducing surgery ($p = 0.01$).

Conclusions: This cohort of underserved minority patients demonstrated high uptake of genetic services. Mutation rates and clinical characteristics are consistent with previous reports. More than 50% of eligible *BRCA* mutation carriers underwent a risk-reducing surgery. Further studies are needed to better understand uptake of risk management protocols. Long-term follow-up will allow for exploration of psychosocial and cultural influencers in an underserved ethnically diverse setting.

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EDUCATION AND COMMUNICATION

P039

Anger, Frustration, and Regret Among Women with a Strong Family History of Breast/Ovarian Carcinoma Who Subsequent to Their Breast Cancer Diagnosis Tested *BRCA*-Positive

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Objective: To explore feelings of early-onset breast cancer patients with strong family histories of breast/ovarian cancer who learned about their hereditary risk after their diagnosis and eventually tested positive for a *BRCA* gene mutation.

Methodology: Women diagnosed with breast cancer less than 52 years of age, who subsequently learned that their significant family history of cancer would have qualified them for genetic counselling and testing, and eventually tested positive for a *BRCA* gene mutation were identified. The 34 women deemed eligible for this study were sent a letter of information, consent form, and questionnaire by their primary genetics provider. Upon receipt of subjects' completed questionnaires, mixed-methods analyses was performed using grounded-theory-based qualitative and quantitative analyses.

Results: Narratives for qualitative analysis were received from 13 women (13/34, 38% participation). Four themes emerged: I–types of emotions; II–emotional response; III–coping with emotions; IV–advice to other women at similar risk. Women felt they should have learned about their risk from their family physician and/or through public education. A timeline of emotions revealed that anger, frustration, and regret were experienced after receiving their *BRCA* gene test results, but were not at the time of their cancer diagnosis.

Conclusion: Anger, frustration, and regret arose from the denied opportunity for genetic counselling, testing, and increased surveillance options in women who learned subsequent to their diagnosis that their early-onset breast cancer was linked to their family history of breast/ovarian cancer. With increased public and physician education, it is hoped that women with significant family histories of breast/ovarian cancer will be identified prior to diagnosis, and given options regarding earlier cancer surveillance and risk reduction strategies. Physicians have an ethical and legal responsibility to take a current family history of cancer and offer referral to all women and men at potential increased risk for genetic counselling and risk assessment.

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P041

Centre ROSE: Support and Information Resources for Families at High Risk of Hereditary Breast Cancer in Quebec

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Background: In Quebec, psychosocial resources addressing the support and information needs of French-speaking families at high risk of hereditary breast and ovarian cancer (HBOC) are scarce throughout the provincial health care system. For instance, the only *BRCA* genetic testing information materials available in French are from France, which may not be suitable to Quebec's francophone sociocultural context and medical practice. Moreover, at the CHU de Québec, unaffected women do not have access to professional psychosocial support unless they are considering prophylactic mastectomy.

Objective: The Centre ROSE is a 3-year pilot project launched in 2013 aimed at addressing the information and support needs of French-speaking families at high risk of HBOC in Quebec.

Methods: The Centre ROSE's structure is inspired by the Logic Model used in program evaluation. Its activities are fourfold: psychosocial support (individual, group, and peer), information (development of educational materials, documentation centre, lay public conferences), professional education (continuing education, training course), and organizational support. Based at the Centre des maladies du sein Deschênes–Fabia, CHU de Québec, the Centre ROSE seeks to develop collaboration with other clinics throughout the province, particularly in remote areas. All activities are thoroughly evaluated using a variety of methods including validated questionnaires, interviews, focus groups, and administrative data analysis.

Results: This presentation will provide an overview of the content of each activity with their corresponding evaluation process and will include preliminary data. As of January 2014, 828 individuals were registered to the mailing list and receive information about the Centre's activities. Collaboration with a number of clinics throughout the province has already been established.

Conclusion: As a pilot project, the long-term sustainability of the Centre ROSE is challenging. The evaluation of all activities will demonstrate the need to maintain or not these information and support resources for French-speaking individuals at risk of HBOC in Quebec.

P042

Development of a Decision Aid to Help Parents Carrying a *BRCA1/2* Mutation Make Decisions about Communication of Genetic Information to Their Children

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Background: Parents who are *BRCA1/2* mutation carriers are concerned for their children who each have a 50% risk of inheriting the familial mutation. Despite the absence of immediate medical benefits for them, many parents are questioning whether they should share genetic information to their underage children Parents report having trouble deciding when and how to start this kind of discussion. Given the lack of resources available, parents are expressing the need for a tool that can assist them in this process.

Objective: Develop a decision aid to meet the needs of parents carrying a mutation in *BRCA1/2* genes regarding the communication of genetic information to their underage children.

Methods: This is a qualitative study. A prototype of the tool was developed following a literature review of existing tools. It is also based on an analysis of the parents' needs regarding family communication, from our previous studies. This tool was developed in accordance with international standards recommended by the International Patient Decision Aids Standards Collaboration (IPDAS). An advisory committee, composed of health professionals, provided advice and comments on the prototype.

Results: This decision aid is presented in form of a booklet. It includes guidance about children's understanding of heredity and on postponing or disclosing genetic information. It also includes the pros and cons of sharing information, quotes from parents who did and did not communicate such information, specific communication tips, and a personal decision aid worksheet.

Conclusion: The next step will be to improve the decision aid using a focus group approach to incorporate parents' suggestions. Funding will then be sought to conduct a randomized clinical trial to evaluate the benefits to the parents in their decision-making process about sharing genetic information to their underage children.

P044

An Online Tool to Increase Knowledge of Hereditary Ovarian Cancer

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Objectives: 1) To improve knowledge of ovarian cancer. 2) To improve knowledge of the genetic risk for ovarian cancer. 3) To create an innovative online tool to educate women about ovarian cancer

Methods: Ovarian Cancer Canada (OCC) has developed an online strategy to promote knowledge of ovarian cancer and in particular the *BRCA1/2* genetic risk for ovarian cancer. Titled the Knowledge Centre, this user-friendly bilingual portal is housed on the OCC Web site and transfers knowledge about ovarian cancer. This education tool is divided into 4 modules: signs and symptoms; role of genetics; risk factors; and what to do. Recognizing that the public is busy and often nervous to receive information about a fatal disease, the Knowledge Centre was designed to be personable and quick to use. As such, the Centre uses real women to deliver a few minutes of information followed by a short quiz. The module on genetic risk focuses primarily on the *BRCA1/2* gene mutation and reviews the percentage of ovarian cancers that are due to genetic mutations, the *BRCA1/2* mutation, paternal and maternal risk, and which cultural groups are most at risk for this genetic mutation. This portal is positioned prominently on our Web site and promoted in our materials across the country.

Results: Since its launch in 2011, approximately 5400 people have visited the Knowledge Centre. Of those visitors, approximately 35% (1920) have taken the quiz on ovarian cancer. Anecdotal feedback from users has been positive.

Conclusions: The use of this online tool to improve knowledge of the genetic risk for ovarian cancer appears to be successful. In the future, we will more actively promote the Knowledge Centre and link it to more comprehensive information on genetic risk and organizations and resources that can support people believed to be at risk.

EDUCATION AND COMMUNICATION (CONTINUED)

P045

Successful Implementation of an Annual Hospital-Based Conference for *BRCA* Mutation Carriers

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Objective: Since discovery of the *BRCA1* and *BRCA2* genes, great strides have been made in understanding the varied impact of testing positive. Significant challenges remain for *BRCA*-positive patients, which are not always addressed by their medical team. To educate our *BRCA* community regarding advancements in the field and to address the unique medical and psychological needs of *BRCA* carriers, the Beaumont Cancer Genetics Program initiated an annual *BRCA* Symposium.

Methods: *BRCA*-positive individuals and their families from Beaumont Health System and the local community were invited to attend a *BRCA* Symposium initiated in the fall of 2012. A variety of relevant topics were presented, including cancer risks, high-risk surveillance, prophylactic surgery, gynecologic issues, and psychosocial topics. Participants were asked to complete a survey rating the quality, added benefit, and overall impact of the conference at the 2012 and 2013 meetings.

Results: Survey respondents included 48 of 58 participants (83%) in 2012, and 34 of 47 participants (72%) in 2013. Participants included 30 cancer survivors, 23 previvors, and 13 who had a family member who was *BRCA*-positive, but had not tested themselves. The majority were Caucasian (73%), with representation from Asian, African, and Arabic Americans. The primary reasons for attending included gathering information regarding *BRCA*, help with medical decision-making, support, and networking with other individuals who are *BRCA*-positive. 96% of participants felt that the conference met or exceeded their expectations; 88% felt that their knowledge about *BRCA* improved significantly.

Conclusion: This experience demonstrates successful implementation of an annual educational and support symposium for *BRCA*-positive patients and their families. This approach addresses a significant need in this unique population for continued long-term education and support, and serves as a model for others.

P046

A New Intensive Short Training Program of Hereditary Cancer Medical Practice for Genetic Counsellors in Japan

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Objectives: The heredity aspect of cancer has not been taken into full consideration in breast and other cancer clinical practices in Japan. Genetic testing, genetic counselling, chemoprevention, and cancer surveillance and risk-reduction surgeries for high-risk individuals are not covered by the Japanese health insurance system, and a limited number of testing and genetic counselling sessions have been conducted throughout Japan, although the need for these practices is increasing. There have been more than 150 genetic counsellors in Japan, but many of them have not learned enough and not experienced a lot of cancer genetics practice. To improve this situation, we organized an intensive and practical short training program for genetic counsellors.

Methods: A week-long training program was held at Shikoku Cancer Center each year in 2012 and 2013. The curriculum of the program was carefully planned by 3 well-experienced genetic counsellors. The program contains both lectures and practical exercises, including family history intake, pedigree assessment, risk assessment, and supervised role-playing, taught and guided by experienced genetic counsellors, medical doctors, nurses, medical social workers, and a clinical psychologist. Lectures covered clinical oncology, cancer genetics, and all of the practical items needed to conduct a hereditary cancer medical practice. Psychosocial issues and social services were also discussed.

Results and Conclusions: In 2012 and 2013, 7 and 10 people respectively participated in the program. Participants were certified genetic counsellors, nurses, and genetic counselling students. Many participants stated that the content was practical and the whole program was very helpful to make them ready for actual hereditary cancer medical practice. The program organizers also learned the strong and weak points of participants. In this presentation, the program details and the lessons learned by participants and organizers will be described.

ETHICAL–LEGAL ISSUES

P047

Towards an Ethics Safe Harbour for Global Breast Cancer Research

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Biomedical researchers in hereditary breast cancer are becoming increasingly globally connected and collaborative. Though global data-driven research holds great promise for breast cancer discoveries, the underlying research ethics review system in much of the world, including Canada, challenges improvements in human health and paradoxically may not improve respect for persons who participate in research. Case reports illustrate that the current system is costly, fragmented, inefficient, inadequate, and inconsistent. There is an urgent need for criteria to determine whether there is "substantial equivalency" between the principles and procedures, ethics review, and governance to be able to share data across jurisdictions. Building on the international privacy "safe harbour" model that was developed following the adoption of the European Data Protection Directive in 1995, we propose a federated "Safe Harbour Framework for International Ethics Equivalency" that could facilitate the harmonization of ethics review of specific types of data-driven international breast cancer research projects while respecting globally transposable research ethics norms and principles. The Safe Harbour would consist in part of an agency supporting an International Federation for Ethics Review (IFER), formed by a voluntary compact among countries, granting agencies, philanthropies, institutions, and health care, patient advocacy, and research organizations. IFER would be both a central ethics review body and also a forum for review and follow-up of policies concerning ethics norms for international research projects. It would be built on five principle elements: 1) registration, 2) compliance review, 3) recognition, 4) monitoring and enforcement, and 5) public participation. The Safe Harbour would create many benefits for researchers, countries, and the general public. Research participants would enjoy uniform adequate protection, while researchers would enjoy ensured ethics review expertise and a reduction in cost, time, administrative hassle, and redundant regulatory hurdles. Most importantly, society would enjoy the maximization of the potential benefits of breast cancer research.

P048

Ethical Obligation to Provide All Women at High Risk of *BRCA* Gene Mutation-Related Breast/Ovarian Cancer the Opportunity for Genetic Counselling: From "Sarah's Daughters" to Angelina Jolie

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Objectives: To investigate Health Canada's obligation to public and physician education, including why this has been limited regarding hereditary breast/ovarian cancer.

Methods: Using conceptual ethics research methodology, results of two qualitative studies analyzing audience members' comments following performances of *Sarah's Daughters* (>1000 citizens engaged in 9 Canadian cities) were applied to quantitative data on referral patterns to Canadian cancer genetic units following Angelina Jolie "going public" with her breast cancer prevention experience.

Results: Several recent publications indicate physicians lack time to update their genetics education and discuss genetics-related health with patients. In the *Sarah's Daughters*" research (Nisker *et al.* 2006), multiple audience members commented that they saw themselves on stage and became concerned for themselves and their daughters, and angry that their family physician had not brought hereditary breast cancer to their attention. Audience members consistently identified the need for public education. Yet a Southwestern Ontario study (Vanstone *et al.* 2012) found that significant numbers of women continue to learn about the relationship between family history and breast cancer after their breast cancer diagnosis and subsequently test positive for *BRCA* gene mutations. Since Angelina Jolie's public education endeavor, referrals to Canadian cancer genetic units have increased 30%–60%, with many referrals from physicians who had never referred previously. It is not clear whether the public education provided by Angelina Jolie encouraged women to bring their family history to their physician and insist on referral to genetic counselling or informed clinicians.

Conclusions: Many Canadian women at high risk and their physicians were unaware of the association of family history and breast/ovarian cancer until Angelina Jolie's initiative, and some likely remain unaware. Health Canada has the public health obligation to provide public and physician education so that all Canadians at high risk have access to genetic counselling.

ETHICAL–LEGAL ISSUES (CONTINUED)

P049

Analyse anthropologique des politiques de brevetage génétique comme propriété intellectuelle et commerciale au Québec: le cas de *BRCA1/2*

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Objectif : Depuis 2001, les analyses moléculaires des gènes *BRCA1/2* prescrites depuis certaines institutions médicales canadiennes, sont envoyées vers d'autres laboratoires, moins couteux mais aussi permettant de conserver les données génétiques dans leur province ou sur le territoire fédéral. Pour autant, un certain nombre de clinique au Québec se soumette toujours au brevet de Myriad Genetics et ferait appel à la compagnie pour ses diagnostics de *BRCA*.

Cette étude identifie comment la communauté conçoit le rôle des brevets dans les dépistages et diagnostics du cancer et comment les brevets génétiques expriment une culture médicale. On cherche à déterminer comment sont perçus les brevets génétiques par la communauté médicale en analysant et en comparant les variations entre limites idéologiques et limites pratiques.

Méthodologie : La recherche consiste en une analyse anthropologique de la littérature et une étude ethnographique composée de 10–15 participants avec des conseillers génétiques, des médecins, et des juristes de la région de Montréal et de Sherbrooke.

Résultats : Le discours des intervenants de la communauté médicale souligne une équivoque dans la finalité des brevets génétiques. Si ils sont perçus comme une réalité économique du monde scientifique, ils sont néanmoins critiqués dans les lacunes éthiques du partage intellectuel et de la perspective politique de leur nature

Conclusion : Les discours argumentent la volonté d'un changement dans l'accessibilité aux connaissances scientifiques et au partage des données, dans un intérêt éthique et pour un dynamisme politique national. Ces valeurs idéologiques sont cependant dépréciées par la structure commerciale de Myriad qui offre un support technique opérant et efficient aux centres hospitaliers. La brevetabilité des dépistages du *BRCA1/2* par Myriad au Québec renforce les perspectives socioculturelles des biotechnologies, comme valeur positive de performance technocratique, industrielle, qui permet une productivité et un rendement pour les patients.

P050

Abstract: 174 Prelude to "Pigs Fly": The Early History of the Myriad Case

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How and why did the American Civil Liberties Union (ACLU) and the Public Patent Foundation file a lawsuit against Myriad Genetics Corporation in 2009, challenging Myriad's patents on *BRCA1* and *BRCA2* genes?

In June 2013, the Supreme Court handed down its much-heralded ruling in Association for Molecular Pathology *v* Myriad Genetics. The genesis of the case is far less well known. Interviews with the instigators of the case reveal a compelling story about the process of strategically framing patent policy as public interest litigation. The ACLU's initial goal was to raise public awareness about gene patenting. They were not at all certain they would prevail, but believed that at least a case would greatly increase awareness of a perceived problem, and become the focus of public debate ... and possibly policy change. In interviews and background documents, the major players describe how the ACLU was able to build a strong coalition and as a result, beat the odds.

GENOME-WIDE APPROACHES TO IDENTIFY NEW GENETIC RISK FACTORS

P051

Breakpoint Analysis as a Method to Identify Candidate Genes in Ovarian Cancer

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A large proportion of high-grade serous ovarian cancers (HGSCS) display somatic *TP53* mutations and extensive aneuploidy. Though genetic heterogeneity is extensive, nonrandom patterns of structural anomalies suggest that the disrupted regions contain genes important in the etiology of the disease. Previously, we observed that chromosomal breaks in *BRE* in several French Canadian (FC) HGSCS were associated with changes in expression that matched the break locations. Here, we study chromosomal breaks in *HYDIN* which were also observed in >25% of our FC HGSCS.

We used ASCAT to characterize genomic anomalies, heterozygosity, and chromosomal breakpoints in the 532 HGSC samples from the Cancer Genome Atlas genotyped using the Illumina Human Omni1-Quad. For samples with inferred breaks, the effect of the break on gene expression (measured by RNA sequence exon data in 296 samples) was estimated by a mixed model with random effects for each individual, fixed effects for differences in expression across sites within our gene, and individual-specific effects of copy number on expression, comparing before versus after the break.

We identified 87 samples with inferred breaks in *HYDIN.* Breaks tended to cluster towards the 3′ end of the gene. Interestingly, we observed a significant association between expression and copy number within this region in samples with no breaks. Deletion of both copies of the 400 kb region containing the *HYDIN* gene was rare, and exclusively found in samples with breaks. In contrast, loss of a single copy was observed in 151 of the samples without breaks. For the 44 samples with breaks where RNA was available, our mixed-effects model identified 4 samples in which there appeared to be a statistically significant change in expression associated with the location of the breakpoint ($p < 0.05$).

In conclusion, we bring stronger evidence to warrant consideration of breakpoints for identifying genes implicated in HGSC.

P052

Targeted Next-Gen Sequencing in High-Risk *BRCA1*- and *BRCA2*-Negative Breast Cancer Patients

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Breast cancer (BC) is the most common cancer type also in the Czech Republic. We have screened 1035 high-risk breast/ovarian cancer patients and identified 269 carriers of pathogenic variants in 8 breast cancer BC-susceptibility genes (*BRCA1*, *BRCA2*, *TP53, PALB2*, *CHEK2, ATM, NBN,* and *PPM1D*). The most frequent alterations in identified mutation carriers were pathogenic variants in *BRCA1* ($n = 188$); however, we failed to identify some pathogenic variant in 74% of analyzed high-risk patients.

To identify underlying BC-susceptibility variant in the largest HBC patient subgroup, we launched a next-gen sequencing (NGS) project targeting 594 genes that code for proteins involved in DNA repair or influencing BC pathogenesis using custom sequence capture panel and SOLiD sequencing. The NGS analysis has been performed in 338 high-risk patients and 145 non-cancer controls so far. The preliminary data reveal presence of frameshift, stop-gain, or splicing variants in high-risk BC patients that were confirmed by Sanger sequencing. These variants affect several interesting genes, including the genes coding for Fanconi anemia proteins or proteins involved in DNA repair pathways (for example, Wrn, Brip1, Rad18); however, we also identified 2 *BRCA1* mutations and 1 *BRCA2* mutation affecting the conservative splicing sites that has not been detected by mutation analysis performed previously.

Despite the entire study being ongoing and the identification and classification of found SNPS being currently under investigation, our preliminary data indicate that targeted NGS, using panels of BC-susceptibility genes tailored to the analyzed geographic population is a rational, powerful, and economic strategy surpassing the classical strategies of mutation analysis. However, the implementation of exome-wide or genome-wide NGS approaches will be indispensable for the detection of population-specific rare variants or private mutations in high-risk individuals.

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MOLECULAR AND CELLULAR BIOLOGY OF HEREDITARY CANCERS

P053

The Australian Familial Male Breast Cancer Study: Somatic Copy Number Variant Analysis

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Objectives: Male breast cancer (MBC) is a rare, easily recognized subtype of breast cancer, whose risk factors include family history, sex hormone imbalance, heritable mutations in FBC predisposition genes, most notably *BRCA2*, and sex chromosome anomalies such as Klinefelter syndrome. The causes and molecular basis of MBC are not well elucidated, although the majority of cases (~85%) are invasive ductal carcinoma of luminal A subtype based on immunopathologic indices. Current treatment regimens are predicated on results extrapolated from female breast cancer studies.

Methods: Collaboration between the Australian Familial cancer centres and K-Confab Breast cancer foundation is currently unpinning recruitment of *BRCAx* MBC cases and their families with clinical and biologic data into an Australian MBC cohort study. As part of this study, genome-wide, somatic copy number variant (CNV) analysis is undertaken on DNA microdissected from well-annotated FFPE MBC tumour blocks using a 330 K molecular inverse probe set, Oncoscan 2.0 (Affymetric). Data are analyzed using Nexus copy number 7.0 Discovery Edition and Partek Genomic Suite 6.5.

Results: Here, we present the CNV data from the first 20 *BRCAx* MBC cases compared to published datasets. Of those cases, 25% displayed a stable genome with less than 7% of the genome displaying LOH, and fewer than 25 regions of CNV, with no areas of high copy number gain. There is no correlation with age/grade between these cases and the remaining cases, which demonstrate whole-arm copy number gain and loss with infrequent regions of high copy number gains. Many FBC-associated CNV leitmotifs were present, as well as unique MBC-specific CNVs. Amplification of *FGRF1* and *CCND1* was commonly identified.

Conclusions: Increasing evidence indicates that MBC forms a heterogeneous group of disorders despite its relatively homogenous clinical immunopathologic presentation. Further study is required to provide a more evidence-based rationale for its most effective treatment.

P055

BRCA and Early Events in the Development of Serous Ovarian Cancer

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Objectives: Women who have an inherited mutation in the *BRCA1* gene have a substantial increased lifetime risk of developing the most common and most aggressive histotype of epithelial ovarian cancer, high-grade serous carcinoma. The mechanisms underlying malignant transformation in these estrogen-responsive tissues are poorly understood, but likely involve loss of heterozygosity of the remaining wild-type *BRCA* allele in addition to inactivation of p53. In normal cells of mutation carriers, only one allele is mutated, and *BRCA1* function is presumed to be intact. This may however not be true, as evidence in support of *BRCA1* haploinsufficiency accumulates. This study proposes to identify genes which may predispose tubal epithelium in heterozygotes to malignant transformation, and to understand the effect of the ovulatory cycle on gene expression, inflammatory infiltrate subpopulations, and epithelial proliferation in histologically normal but high-risk tubal epithelium.

Methods: Using snap-frozen and paraffin-embedded formalin-fixed human tubes obtained from *BRCA1* mutation carriers undergoing RRSO, women undergoing salpingectomy for non-malignant reasons, and women undergoing debulking surgery for high-grade serous carcinoma with known germline mutations, we analyzed gene expression profiles and immunoprofiles of tubal epithelium, using cases microdissected for gene expression analyses were controlled for age and ovarian cycle status.

Results: *BRCA1* MRNA levels were not substantially different between carriers and non-carriers. *BRCA1* mutation in morphologically normal fallopian tube epithelium confers a significantly altered gene expression signature, with altered pathways, including the TGF-β pathway, MAP kinase pathway, the adipokine signalling pathway, inflammation, and the p53 signalling pathway. In particular, genes involved in DNA damage and inflammation were validated as both having transcriptional and translational differential expression in the normal fallopian tubes (ampulla and fimbria) of *BRCA* mutation carriers.

Conclusions: It is known that factors related to parity, ovulation, and hormone regulation have a dramatic effect on the risk of developing ovarian cancer in both *BRCA* mutation carriers and non-carriers. Recently, we have shown that the transcriptional profile of histologically normal fallopian tube epithelia of *BRCA* mutation carriers is different from wild-type FTE and possibly hints at *BRCA* haploinsufficiency. We propose that changes of the transcriptome in *BRCA* mutation carriers reflect an altered response to the microenvironment ovulatory stresses, which may include altered reproductive hormone levels and the ovulatory inflammatory response.

MOLECULAR PATHOLOGY AND GENETIC ANALYSES OF *BRCA1/2*–ASSOCIATED CANCERS

P057

The Effect of Physical Activity and Body Size on *BRCA1* Expression

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Background: The incomplete penetrance associated with a *BRCA1* mutation suggests that non-genetic factors may play a role. Various reproductive factors influence breast cancer risk; however, the role of diet and lifestyle, including body size and physical activity, is not known. It is believed that a haploinsufficient state is responsible for the predisposition to cancer among *BRCA1* mutation carriers. Thus, factors which might increase the physiologic expression of the normal copy of *BRCA1* and normalize protein levels may mitigate the effect of the mutation. Two recent studies reported increased *BRCA1* expression in subjects with regular physical activity. Whether this is true in women with a *BRCA1* mutation is not known.

Objectives: The objective of this study is to evaluate whether physical activity and/or body size correlate with *BRCA1* mRNA and protein expression.

Methods: Women (n = 55) will be asked to wear the GT3X accelerometer for 7 days, a triaxial monitor that detects acceleration and converts these data into measures of physical activity. Anthropometric measurements (that is, weight, height) will be obtained, and medical and lifestyle information will be collected via questionnaire. Blood samples will be collected after 7 days for RNA and white blood cell isolation. *BRCA1* mRNA will be quantified using the Nanostring nCounter Analysis System, and *BRCA1* protein levels will be confirmed using Western blot in samples corresponding to the 1st and 5th quintile of mRNA expression. Linear regression will be used to examine the relationship between levels of physical activity and body size with *BRCA1* mRNA and protein expression levels. Should findings from this study provide evidence for a relationship between physical activity or body size and *BRCA1* expression, this will support the development of lifestyle prevention options leading to a decrease in the number of cancer cases attributed to a *BRCA1* mutation. Results are expected in February 2014.

P058

The Sensitivity of Ovarian Cancer Cells to an Insulin-like Growth Factor Receptor Kinase Inhibitor Correlates with the Functionality of the Homologous Recombination Repair

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Prior data from our lab suggests that ovarian cancers (OCs) bearing dysfunctional homologous recombination repair (HRR) might be more sensitive to insulin-like growth factor 1 (IGF-1) targeting.

Objective: To evaluate the correlation between the HRR function of OC cells and their sensitivity to IGF-1 receptor kinase inhibitor (IGF-1Rki: BMS-536924).

Methods: OVCAR3, SKOV3 (wild-type *BRCA1*), IGROV1 (heterozygous *BRCA1*), A1847 (methylated *BRCA1*), and UWB1.289 (mutated *BRCA1*) were evaluated for HRR status using RAD51 foci formation induced by cisplatin using immunofluorescence. The LC_{50} for BMS-536924 of these cell lines was assessed by clonogenic survival assays. Western immunoblotting was performed to determine the expression of the IGF-1R pathway. Finally, we developed primary cell lines from patient tumours and performed similar analysis.

Results: Cells with mutated or methylated *BRCA1* showed impaired HRR function, determined by reduced RAD51 foci formation, as compared to cells with wild-type and heterozygous *BRCA1*. Mutated or methylated *BRCA1* cells also show elevated expression of p-S6, p-AKT, p-IRS suggesting an overactivation of the IGF-1R pathway as compared to cells with proficient HRR function. HR-deficient cell lines (UWB1.289 and A1847) had lower LC_{50} to anti–IGF-1 inhibition by BMS-536924 compared to HR-proficient cell lines (SKOV3, OVCAR3, and IGROV1) suggesting a correlation between HRR function and sensitivity to IGF-1R inhibitors in OC cells. Primary cell lines with impaired HRR also showed lower LC_{50}.

Conclusion: OC cells harbouring deficient HRR are more sensitive to IGF-1Rki. Given the significant proportion (up to 50%) of ovarian cancers with impaired *BRCA1/2* function, the strategy of targeting IGF-1R might lead to improved personalized therapeutic regimens in these patients.

P059

A Robust Method for Next-Generation Sequencing Tumour *BRCA1* and *BRCA2* Analysis in Formalin-Fixed Paraffin-Embedded Tissue

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Germline mutations in *BRCA1* or *BRCA2* lead to a high lifetime probability of developing ovarian or breast cancer in women. Researchers have established that these genes can also be involved in the development of nonhereditary tumours, as a proportion of ovarian and breast cancers contain somatic (tumour-only) *BRCA1/2* mutations. As these patients may benefit from treatment with poly ADP ribose polymerase (PARP) inhibitors, it is important to be able to test for *BRCA1/2* mutations in routinely available tumour samples.

Tumour samples available for testing are typically formalin-fixed paraffin-embedded (FFPE), in which the DNA tends to be limited in quantity and fragmented, making the analysis of large genes such as *BRCA1* and *BRCA2* extremely challenging. This is made more difficult as somatic mutations may be represented in only part of the test sample, due to the presence of infiltrating stroma and surrounding normal tissue.

Using DNA samples extracted from 40-micron-thick FFPE ovarian (n = 68) and breast (n = 30) cancer specimens, we investigated the utility of the GeneRead DNAseq Targeted Exon Enrichment Breast Panel (Qiagen) using the *BRCA1/2* primer pools only, followed by library preparation and adaptor ligation using the TruSeq DNA PCR-Free HT Sample Preparation Kit (Illumina) and 2×150 bp next-generation sequencing (NGS) analysis on the MiSeq (Illumina). Following optimization of the method, we successfully analyzed more than 84% (83/98) of samples with >90% coverage of *BRCA1/2* coding regions at a minimum of 100× read depth. All *BRCA* mutations identified were confirmed where possible by Sanger sequencing or replication to eliminate the risk of false-positive results due to FFPE artefacts. It was also determined that *BRCA1/2* mutations could be detected if present in >10% of the sample DNA.

Routine analysis of tumours for *BRCA1/2* mutations is feasible and could be performed prospectively to inform future treatment for ovarian or breast cancer patients.

P061

A Rainbow of Genetic Diversity in the South African Population Complicates *BRCA* Testing

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Objectives: South Africa (SA) is a heterogeneous nation consisting of multiple genetically unique population groups including the African San, various Bantu-speaking tribes, the SA Indian, and the Afrikaner with its European heritage. Although limited diagnostic testing for *BRCA* mutations commenced in 2002, testing remains challenging. We report on the progress made over the last decade.

Methods: *BRCA* diagnostic requests were received for 1120 SA breast and/or ovarian cancer patients. The extent of the family history varied from young single affected (dx 24–45) to elderly females (dx >50) with multiple affected family members. Of these, 46.3% was Afrikaners, 6.4% black African, 4.3% SA Indian, and 16.9% of mixed ancestry.

Results: Of those tested, 18% carried a deleterious mutation. *BRCA1* mutations accounted for 26% compared to 74% for *BRCA2*. Collectively, the Afrikaner founder mutations accounted for 21.2% of the Afrikaner patients with their European heritage. The founder mutation (*BRCA2* c.5771_5774del,p.Ile1924ArgfsX38) detected for the Xhosa and mixed ancestry (African San, African non-San, European, South and East Asian heritage) populations were detected in 2.2% and 5.3% respectively. A second recurrent mutation was identified in 5 patients of mixed ancestry. The positive detection rate obtained for the limited number of Zulu and Sotho/Tswana patients tested, was extremely low (average 7.14%), with no recurrent mutations detected. The Indian patients had the highest occurrence of deleterious mutations (17.8% of 42 patients), with only a single recurring mutation detected for 3 apparently unrelated families.

Conclusion: Diagnostic testing within the rainbow nation is complicated due to the large extent of genetic diversity present, which is reflected in the diverse mutational spectrum observed. Additional research is needed as currently limited information is available for the majority of SA citizens, namely the black Bantu-speaking tribes. Additional data will contribute to better diagnostic testing and clinical management of these patients in future.

MOLECULAR PATHOLOGY AND GENETIC ANALYSES OF *BRCA1/2*–ASSOCIATED CANCERS (CONTINUED)

P062

SET Features and Tubal Intraepithelial Carcinoma in Women With and Without *BRCA* Mutations

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Background: Extrauterine high-grade serous carcinoma (HGSC) has been linked to the distal fallopian tube via an early preinvasive intraepithelial neoplasm (TIC). TIC is found in approximately 5% of prophylactic salpingo-oophorectomies of women with *BRCA1* or *BRCA2* mutations (*BRCA*+) and 40% of unselected HGSCs. We recently discovered that TICs are paradoxically less commonly found with high-stage HGSC, raising questions about the origin of many HGSCs in this population. We sought to validate this discovery in more than one context by correlated specific tumour patterns with TIC frequency.

Design: A series of documented *BRCA*+ and *BRCA*– HGSCs that were analyzed by the SEE-FIM protocol were scored for 1) associated TIC and 2) pattern of serous carcinoma differentiation, including SET features (solid, pseudo-endometrioid, transitional) and classic serous differentiation. Ovaries from a separate subset of tumours lacking TIC were analyzed by calretinin staining to confirm or exclude neoplastic transformation of the ovarian surface epithelium (OSE).

Results: Of 24 and 30 *BRCA*+ and *BRCA*– cases were studied; 5 (21%) and 16 (53%) respectively were TIC+ ($p = 0.02$). Of 20 and 28 informative for optimal histologic classification, 9 (45%) and 2 (7%) were SET+ ($p = 0.004$). Of 4 *BRCA*+ TIC+ cases, 1 was SET+, and of 14 TIC+ *BRCA*– cases, none were SET+. Overall, 17 of 18 (94%) TIC+ cases showed classic serous differentiation versus 20 of 30 (67%) TIC– cases ($p = 0.035$). Analysis of 100 ovarian histologic sections from 25 additional TIC– cases did not reveal evidence of transformed OSE in proximity to the neoplasm.

Conclusion: SET features correlate both with *BRCA*+ and the absence of TIC, the latter finding paradoxically linked to high-stage HGSCs in *BRCA*+ women. The origin (tube vs. ovary) of this significant subset of HGSCs remains unclear, but resolution of this question is particularly relevant to the *BRCA*+ population if prophylactic salpingectomy alone is contemplated as a cancer prevention measure. There is no immunohistochemical evidence from this analysis to implicate a direct transformation of the ovarian surface epithelium.

P063

Carcinosarcoma of the Ovary in a Patient with a Germline Mutation in *BRCA1*: A Case Report

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Women with germline mutations in *BRCA1* and *BRCA2* have an increased risk of developing breast and/or ovarian cancers. The most common type of ovarian cancer associated with pathogenic mutations in *BRCA1* and *BRCA2* are epithelial ovarian cancers, the most common being high-grade serous tumours. Recent evidence suggests that a portion of these ovarian cancers derives from the epithelium of the fallopian tube. Historically, genetic testing for *BRCA1* and *BRCA2* has been preferentially offered to patients with high-grade serous ovarian cancers alone; however, germline mutations in *BRCA1* and *BRCA2* have also been shown to increase the chance for other types of ovarian cancers. Ovarian carcinosarcomas (malignant mixed mesodermal tumour) are a rare type of malignant epithelial ovarian cancer. Carcinosarcomas of the ovary contain histologically malignant epithelial and mesenchymal elements. Diagnosis of ovarian carcinosarcomas typically occurs in postmenopausal women at advanced stage and is associated with a poor prognosis.

Here we describe the case of a 60-year-old woman who was diagnosed with an ovarian carcinosarcoma with a serous carcinomatous component, and a concurrent fallopian tube *in situ* and invasive serous carcinoma. The patient was found to carry a germline mutation in *BRCA1* (c.4485-2A>G). To the best of our knowledge, this case represents the second instance of an ovarian carcinosarcoma in association with a pathogenic germline mutation in *BRCA1*. We will describe the patient's family history, the tumour pathology, course and treatment, as well as connect the *BRCA1* mutation to the pathogenesis of this tumour. We recommend that genetic testing for *BRCA1* and *BRCA2* is offered to individuals who have been diagnosed with ovarian cancer histologies other than high-grade serous tumours in the context of a suggestive family history for hereditary breast and ovarian cancer.

P064

Therapeutic Approaches to *BRCA*-Associated Pancreatic Cancer

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Pancreatic adenocarcinoma (PC) is the 4th leading cause of cancer death in Canada and remains one of the most lethal cancers. These daunting treatment failures are largely due to ineffective chemotherapeutic regimens. The current era of genetic cataloguing of common cancers promises to identify subsets of patients who will benefit from tailored treatments. As with many common cancers, a fraction of PC has a genetic predisposition, including a subset caused by germline mutations in the *BRCA1/2* genes. Recent observations by our group and others have demonstrated remarkable therapeutic responses of *BRCA*-associated PC to treatment regimens including platinum-based agents and poly ADP-ribose polymerase inhibitors (PARPis), which target DNA repair defects in these tumours. To identify optimal treatment regimens for *BRCA*-associated PC, we performed a head-to-head comparison of these agents. *In vitro* cytotoxicity assays comparing gemcitabine, cisplatin, oxaliplatin, carboplatin, and veliparib (PARPi), in *BRCA2*-intact (MIA PaCa-2) and -deficient (Capan-1) PC cell lines, confirm that *BRCA2*-deficient (Capan-1) cells are more sensitive than *BRCA2*-intact (MIA PaCa-2) cells to platinums and PARPis, indicated by a 4-fold increased sensitivity to cisplatin and oxaliplatin, and a 7-fold increased sensitivity to veliparib in *BRCA*-deficient PC cells. To determine if chemosensitivity to PARPis can be induced in PC without inherent *BRCA2* inactivation, we reduced *BRCA2* expression in *BRCA*-proficient PC cell lines using shRNA constructs targeting *BRCA2*. We show impaired DNA repair by homologous recombination in the *BRCA2* knockdown PC cell lines as indicated by a reduction in nuclear Rad51 and gamma-H2AX foci. However, chemosensitivity to PARPi was not inducible in the *BRCA2* knockdown PC cell lines. In summary, we compared the cytotoxicities of agents with potential therapeutic advantages in *BRCA*-associated PC, which we are now validating *in vivo* using patient-derived xenografts. In addition, our data suggest that reduction in *BRCA2* expression is not sufficient to induce PARPi sensitivity in PC without inherent *BRCA2* inactivation.

P065

Gene Expression Profile in Ovarian Stroma of *BRCA1* Mutation Carriers and Age-Matched *BRCA1/2* Wild-Type Controls

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Background: Haploinsufficiency of *BRCA1* leads to alterations in the gene expression profile that can occur prior to genomic and histological signs of malignant transformation. Although most of high-grade serous carcinomas arise from the serous epithelium of the fallopian tubes, tumour growth and progression is preferential to the ovary, where the effects of ovulation on the microenvironment may contribute to carcinogenesis. In *BRCA1* mutation carriers, ovarian stroma may play an important role in initiation and promotion of carcinogenesis.

Objective: To determine the gene expression profile in ovarian stroma of *BRCA1* mutation carriers and age-matched *BRCA1/2* wild type controls and to identify alterations in pathways related to carcinogenesis.

Materials and Methods: After IRB approval, the Gilda Radner Hereditary Cancer Program's registry will be reviewed. Twelve premenopausal *BRCA1* mutation carriers, age 35–50 years, who underwent risk-reducing salpingo-oophorectomy and had histopathologically normal fallopian tubes and ovaries, and 12 *BRCA1/2* wild-type age-matched controls who underwent salpingo-oophorectomy for non-oncologic indications will be included. Clinical modifiers of the risk of serous carcinoma such as parity, history of infertility, and OCP use will be analyzed. Ovarian stroma will be isolated from the FFPE. RNA will be isolated and sequenced. Sequencing reads will be aligned. The Benjamini–Hochberg step-up method will be applied to control FDR. The list of differentially expressed genes will be generated based on statistical (FDR < 0.2) and biologic significance (>2-fold change).

Anticipated Results: The study will provide the proof of principle for identification of the gene expression profile in ovarian stroma of *BRCA1* carriers and age-matched *BRCA1/2* wild-type controls. In *BRCA1* carriers, alterations in expression profile of the genes that participate in DNA repair and proinflammatory signalling are expected—specifically, changes associated with impaired DNA double-strand breaks repair and activation of cell senescence program.

NON-*BRCA1/2* GENETIC FACTORS ASSOCIATED WITH CANCER RISK

P066

High Accuracy and Expanded Yield from Next-Generation Testing of Multiple Cancer Risk Genes

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Objective: Gene panels assayed using next-generation sequencing (NGS) technologies are moving from research labs into clinical use, with the potential to provide improved diagnostic yield rapidly and at low cost. It is important to understand the performance of these tests in detail by comparison with traditional sequential tests, and to articulate the differences between research and clinical uses of NGS.

Methods: 712 Germline DNA samples were collected: a) 600 from 2 clinical cancer centres, all previously tested for *BRCA1* and/or *BRCA2* using traditional methods; b) 112 reference samples from public biobanks, chosen both to have broad coverage of genes previously tested and also enriched for variants known to be challenging for NGS (for example large indels and small copy-number deletions and duplications). These were blinded and tested on a custom NGS panel including 27 hereditary cancer genes with laboratory protocols and bioinformatics methods specifically adapted for clinical use. The NGS and previous results were compared and, for the very few discrepancies initially observed, samples were sent to a third-party laboratory for resolution.

Results: 100% analytical sensitivity and 100% analytical specificity were observed between NGS and all confirmed previous results, including sequence and copy-number changes. Clinical interpretations were also highly concordant, although with a slightly higher rate of VUS (variant of unknown significance) in the NGS data (6% vs. 4%). This was expected from following ACMG (American College of Medical Genetics) classification guidelines using public, and not proprietary reference databases. Roughly 40% more cases saw a validated positive reportable finding with the NGS panel.

Conclusion: NGS, when carefully applied, can produce high-quality clinical results compared to traditional testing. The high specificity observed suggests that it may become possible, in some circumstances, to remove the need for orthogonal confirmation and further improve time to diagnosis and reduce costs using NGS.

P067

RAD51C Mutations in Austrian Breast and Ovarian Cancer Families

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Objectives: Mutations in the repair gene *RAD51C* are associated with a significantly increased risk for breast and ovarian cancer. Their low prevalence could be owed to the fact that none of the previous studies has investigated the presence of large rearrangements. We have analyzed *RAD51C* mutations in familial breast and ovarian cancer by using both sequencing and MLPA (multiplex ligation-dependent probe amplification) analysis.

Methods: Direct sequencing and MLPA analysis of *RAD51C* was performed on DNA from 907 non-*BRCA*-mutation carriers. At the time of analysis, 500 patients (55%) had developed breast and/or ovarian cancer, while 407 (45%) had remained healthy. A familial breast cancer background was present in 587 probands (65%), while 159 (18%) came from families with breast and ovarian cancer. A familial ovarian cancer background was present in 27 (3%). In addition, DNA from 101 healthy women and from 159 *BRCA1* and 76 *BRCA2* mutation carriers was also analyzed. The pathogenicity of the variations was evaluated using *in silico* and *in vitro* methods.

Results: 17 Genetic variations were found in the overall population: 2 variations were within the 5′UTR, 1 within the 3′UTR. Intronic (n = 4), missense (n = 6), frameshift (n = 1), and synonymous (n = 3) variants were also found. Of these, c.224_225insA and c.905-2delA, both detected in families with ovarian cancer cases, were classified as definitively pathogenic. A third variant, found in a breast cancer–only family, is probably pathogenic (c.428A>G). MLPA did not identify a single large rearrangement in any of the 907 samples investigated.

Conclusions: The overall frequency of *RAD51C* mutations in our high-risk population was 0.2%. In families with a history of ovarian cancer, the prevalence of pathogenic mutations (1%) suggests a potential role for routine *RAD51C* analysis in this population, while MLPA does not offer an additional diagnostic benefit.

P068

Common Genomic Variants Are Associated with Incidence and Clinicopathogenic Features in Familial Breast Cancer.

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Objectives: Large-scale genome-wide association studies (GWAS) have identified more than 70 common genomic variants associated with breast cancer. It is estimated that these variants account for ~28% of the familial risk of breast cancer. We assessed the association of recently described variants with breast cancer cases from high-risk breast and/or ovarian cancer families. In addition, we investigated the association of these variants with clinicopathogenic features, including hormonal status and grade of tumour differentiation within our highly characterized cohort.

Methods: We examined 1136 female index cases from the Australian Victorian familial breast and ovarian cancer cohort. All individuals were assessed as high-risk through genetic testing services and had completed genetic screening for *BRCA1* and *BRCA2* mutations. High throughput genotyping was performed using the Fluidigm system for 62 common variants identified in GWAS (success rate > 0.95). Control genotype data was obtained from an Australian population-based control group (n = 711). Detailed breast cancer pathology was reviewed for 400 of the cases.

Results: Individual associations in the familial setting were stronger compared to previous breast cancer GWAS. A significantly higher number of risk variants were found among women who did not harbour a *BRCA1* or *BRCA2* mutation when compared to population controls ($p = 1.35\times10^{-39}$) and those who did harbour a *BRCA* mutation (p = 0.0004). Women in the top quartile based on the number of risk variants had an average relative risk of 2.3. Many of the variants showed significant associations with hormone status and grade.

Conclusions: Results of this study confirm the important contribution of common variants to the incidence of breast cancer in the familial setting. The data indicate a direction for further research to determine if these associations can be used to help understand tumour heterogeneity and lead to improved treatment and prevention for high-risk breast and ovarian cancer families.

P069

Analyses of Genome-Wide Linkage Scan Data Among Families with Aggregation of Breast and Prostate Cancer Reveals Evidence for Linkage at 16q21-23

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Objectives: Epidemiologic studies have shown a co-clustering of breast and prostate cancer suggesting that there are germline variants that increase the risk of both hormonally driven neoplasms. Mutations in *BRCA1* and *BRCA2* genes may explain a small portion of the observed co-occurrence of breast and prostate cancer within families. The current investigation focuses on the delineation of chromosomal regions which may harbour new genes that play a role in the aggregation of breast and prostate cancer among first-degree family members.

Methods: A genome-wide linkage scan was conducted on 50 families participating in the University of Michigan Prostate Cancer Genetics Project. All families had at least 2 first-degree relatives diagnosed with prostate cancer and at least 1 female relative diagnosed with breast cancer in a first-degree relationship with one of the participating prostate cancer cases. Genome-wide multipoint nonparametric linkage analyses for the combined phenotype of prostate and breast cancer were performed using the software Merlin.

Results: The strongest evidence for linkage was detected at 16q22 (LOD: 3.07 at rs722579), a region previously reported to be in linked to prostate cancer. This region contains several interesting candidate genes including known prostate cancer tumour suppressor genes *CDH1, WWOX,* and *ATBF1,* as well as *BCAR1,* a gene involved in a number of critical carcinogenic processes including cell migration, growth, and differentiation.

Conclusions: Next-generation sequencing of the genes in this region in our linked families are in progress to identify new mutations that explain clustering of prostate and breast cancer in these families and provide new information on shared genetic pathways between these two common cancers.

NON-*BRCA1/2* GENETIC FACTORS ASSOCIATED WITH CANCER RISK (CONTINUED)

P070

The Prevalence of *ATM* Sequence Variants in a Belgian Breast Cancer Population

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ATM has been recognized as a breast cancer susceptibility gene. However, there is no consensus on the associated risks for heterozygous carriers. This may depend on the type of sequence variant, the position in the gene or the functional effect (haploinsufficiency versus dominant negative effect).

We investigated the complete coding region and splice sites of *ATM* in 185 unrelated female and 110 male breast cancer patients. We compared the prevalence of heterozygous potentially deleterious variants in 190 healthy controls. The female breast cancer patients were investigated by high-resolution melting, followed by Sanger sequencing of the aberrant melting curves. The male breast cancer patients and controls were sequenced on the MiSeq platform.

We detected 4 truncating and 2 splice-site mutations in the female breast cancer population (6/185, 3.2%) and 3 splice site mutations in 110 male breast cancer patients (2.7%). A 1% (2/190) prevalence of deleterious mutations was detected in the control individuals. Additionally, 32 different missense variants were detected in the female and male breast cancer population, of which 12/32 were predicted to be pathogenic by Alamut prediction software. The missense variants were detected with a comparable prevalence in the cases versus the healthy controls, including missense mutations in the functional domains at the 3′ end of the gene. Unfortunately, our study does not have sufficient power to estimate the risks associated with different variants.

This study provides strong evidence for a role of *ATM* sequence variants in both male and female breast cancer. Further analyses using the G2 micronucleus test (Claes *et al.,* 2013) will be performed for the missense variants to distinguish neutral from more likely pathogenic variants.

P071

Consideration of Expanding the Testing Criteria for *TP53* Testing to Include Women with Breast Cancer >35 Years of Age

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Li–Fraumeni syndrome (LFS) has been associated with early-onset breast cancer, and more than half of these families have mutations in the tumour suppressor gene *TP53*. The National Comprehensive Cancer Network (NCCN) recently increased the early-onset breast cancer testing criterion in its *TP53* testing guideline from under age 30 to under age 36. We aimed to study whether individuals with a *TP53* mutation identified by either single gene testing or by multigene panels met NCCN testing criteria and how the age of diagnosis of breast cancer differs in the two groups. From our total of 9578 patients who underwent testing for *TP53*, 146 individuals were identified to carry a pathogenic mutation in the *TP53* gene. Of these, 79.5% (116/146) were identified by single-gene testing and 20.5% (30/146) were identified by multigene testing. Of the 116 mutation-positive patients identified through single-gene testing, 21% (25/116) did not meet NCCN diagnostic/testing criteria for LFS, including 10 individuals with a personal history of breast cancer over age 35. Of the *TP53* mutation–positive patients identified by multigene panels, 53% (16/30) did not meet NCCN diagnostic/testing criteria for LFS, including 9 individuals with a personal history of breast cancer diagnosed over age 35. In addition, 21% (31/146) of the 146 mutation-positive patients carried a gross deletion or duplication which would not have been identified by *TP53* sequencing only, highlighting the importance of including a companion dosage method for truly comprehensive *TP53* testing. These results demonstrate that a substantial portion of mutation carriers will be missed if testing is restricted to individuals meeting established criteria for *TP53* testing. Based on our data, broadening testing criteria to include women diagnosed with breast cancer over 35 years of age would result in the identification of more individuals/families at high cancer risk due to germline *TP53* mutations.

P072

Association of *TP53* with Breast Cancer Risk in Moroccan Patients

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Objective: *TP53* gene is a tumour suppressor gene and plays an important role in cell cycle progression control, DNA damage repair, genomic stability, senescence, and apoptosis. Some polymorphisms in this gene have been associated with the development of a number of cancers including breast carcinoma. PIN3 Ins16bp polymorphism has been widely studied in different populations for an association with breast cancer risk. However, studies have yielded conflicting results.

Methods: DNA samples of 105 patients with confirmed breast cancer and 114 healthy controls were analyzed.

Results: The genotype frequencies were 69.5% for wild-type homozygous (A1A1), 26.7% for heterozygous (A1A2), and 3.8% for mutated homozygous (A2A2) in breast cancer cases. In healthy controls, we observed 68.4%, 24.6%, and 7% for wild-type homozygous (A1A1), heterozygous (A1A2), and mutated homozygous (A2A2) respectively. No statistically significant association was observed between Moroccan patients and controls with an odds ratio of 1.07 (CI: 0.58 to 1.97; $p = 0.83$) for the heterozygous A1A2 and 0.53 (CI: 0.15 to 1.85; $p = 0.32$) for mutated homozygous A2A2.

Conclusion: Our data suggests that PIN3 Ins16pb polymorphism is not associated with breast cancer risk. However, a limited statistical power because of our small sample size can't be ruled out. A larger future study is needed to confirm our findings.

P073

LEGACY Girls Study (Lessons in Epidemiology and Genetics of Adult Cancer from Youth): Recruitment and Retention Within the Ontario Site

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Background: Studies involving children are often difficult to enrol. The NIH-supported, LEGACY Girls Study has recruited 1040 girls, aged 6–13 years, with and without a family history of breast cancer, across 5 sites (Ontario, California, New York, Philadelphia, and Utah). LEGACY families are being followed for 5 years with data and biospecimen (urine, saliva, blood) collections at 6-month intervals, to determine whether intermediate markers of risk [that is, breast development, age at menarche, breast tissue characteristics measured by experimental optical spectroscopy (OS), and sex and growth hormone levels] and their determinants (that is, prenatal, lifestyle, environmental, hormonal, genetic, and epigenetic factors) are different between girls from very-high- to average-risk families.

Objectives: To enrol 90 Ontario girls with 1 or more first- or second-degree relatives with breast cancer or *BRCA* mutation (cases), and 90 Ontario girls without family history for direct comparison (controls).

Methods: Cases were invited from 4 high-risk genetics clinics surrounding Toronto and from within the Ontario Familial Breast Cancer Registry (OFBCR), a NCI-supported population-based registry. Controls were self-referred through community outreach efforts or friend referrals of enrolled participants. To maximize recruitment and retention, the Ontario site created the Junior Scientist Program, offering enrolled girls interactive science experiments at each visit, plus other incentives.

Results: A total of 192 girls (103 cases, 89 controls) were enrolled from high-risk clinics (37%, 71/192), community outreach (27%, 52/192), friend referrals (21%, 40/192), and the OFBCR (15%, 29/192). At baseline, all girls aged 6–13 years (100%, 192/192) provided urine samples, completed anthropometric measures, questionnaires, and gave a blood (26%, 50/192) and/or saliva (71%, 137/192); 90% (82/91) of girls aged 10+ years completed OS. Six months later, 98% (188/192) girls returned for follow-up.

Conclusions: Participants are highly motivated and report enjoying their study experience, expressing willingness to continue until study completion.

NON-*BRCA1/2* GENETIC FACTORS ASSOCIATED WITH CANCER RISK (CONTINUED)

P074

Contribution of Germline *CHEK2* Mutations in Greek Breast Cancer Patients

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Loss-of-function *CHEK2* mutations can be considered quite challenging in respects to clinical decisions. *CHEK2* mutations predispose to breast cancer, as well as to cancer in other organs, while the estimated prevalence of *CHEK2* mutations differs among populations. The lifetime breast cancer risk of *CHEK2* mutation carriers is estimated around 25%, but can be variable between *CHEK2* mutations. Initial screening for mutations in 21 breast cancer susceptibility genes by BROCA panel revealed a high frequency of *CHEK2* variants in Greek breast cancer patients, previously tested negative for *BRCA1* and *BRCA2* mutations. Therefore, a targeted approach was subsequently selected, involving analysis of *CHEK2* exon 3 by Sanger sequencing and *CHEK2* c.1100delC by real-time PCR. The study group consisted of 428 females with family history of breast cancer and 206 females diagnosed with early-onset breast cancer (<45 years) without a reported family history. A high proportion of individuals were found to carry alterations in exon 3 in both groups of patients, namely 3.5% in the first group and 4.8% in the second group. Only 1 patient with reported family history was found to carry *CHEK2* c.1100delC. Additionally, 445 healthy age-matched females were screened for mutations on *CHEK2* exon 3, revealing missense and nonsense alterations in 2.7% of the cases, and 1637 more breast cancer cases were screened for c.1100delC. Three additional c.1100delC carriers were identified, all diagnosed with breast cancer before the age of 50 years, giving a final 0.16% prevalence among Greek breast cancer patients. These data indicate that missense mutations in *CHEK2* exon 3 are quite frequent, with the p.I157T allele being the most common and the *CHEK2* c.1100delC being extremely rare among Greek breast cancer cases.

P075

Next-Generation Sequencing Reveals a Novel Mutation in *MRE11A* Gene in a Family with Hereditary Breast and Ovarian Cancer

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Introduction: The MRN complex consisting of Mre11–Rad50–Nbs1 proteins is involved in *BRCA*-associated double-strand break repair and thereby has a role in maintenance of genomic integrity. Classically, mutations in the *MRE11* gene are associated with ataxia-telangiectasia–like disorder and defects in Nbs1 cause Nijmegen breakage syndrome. Recently, mutations in genes coding for MRN complex have been identified in families with breast and ovarian cancer. However, the extent and impact of their cancer-predisposing effects and their potential clinical value remain unclear. In an effort to identify the genetic mutation in a *BRCA*-negative family with a positive history of breast and ovarian cancer, we used a next-generation sequencing platform to screen all known breast cancer predisposition genes.

Patient and Methods: The proband was a 64-year-old lady diagnosed with breast cancer at 50 years. Her mother had endometrial cancer at age 77, and her maternal aunt had breast cancer at age 79. One of her sister also had breast cancer onset at 60 years. *BRCA1* and 2 gene testing by Sanger sequencing was done in the proband to check germline mutations, followed by MLPA to detect large deletion and duplications. Since no pathogenic change was detected in *BRCA1* and 2 genes, for further evaluation we performed high-throughput next-generation sequencing panel testing for 21 known breast cancer predisposition genes.

Results: A novel heterozygous missense mutation in the *MRE11* gene (NM_005590:c.1090C>T;p.Arg364Ter) was identified. The early termination of amino acid production because of this mutation is expected to affect the protein's function and confer increased risk of breast cancer. The family was counselled regarding carrier screening and increased surveillance.

Conclusion: Our case further highlights the involvement of *MRE11A* gene mutations in conferring increased risk of breast and ovarian cancer and emphasizes the importance of next-generation sequencing both as a cost-effective tool and as an approach to identify novel candidate genes in breast cancer.

P076

Analysis of Large Mutations in *BARD1* Gene in Patients with Breast and/or Ovarian Cancer

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Objectives: Beside *BRCA1/2* genes and several genetic factors associated with hereditary syndromes increasing risk of breast and/or ovarian cancer, a considerable fraction of breast and/or ovarian predisposing factors (>50%) is still unknown. Initial reports indicate that the *BARD1* gene, encoding a protein indispensable for *BRCA1*-mediated tumour suppression function, can be affected by several single-nucleotide mutations predisposing to breast and/or ovarian cancer. Although it was suggested that large mutations (multi-exon deletions or duplications) may contribute substantially to the deleterious variation of *BARD1*, no systematic study of large mutations in *BARD1* was performed so far.

Methods: To elucidate further the role of large mutations in *BARD1,* we designed and generated a multiplex ligation-dependent probe amplification (MLPA) assay covering all exons and flanking sequences of *BARD1*. The assay was designed according to a previously developed strategy using exclusively short, chemically synthesized probes. The MLPA test was used for the analysis of 524 DNA specimens from patients with familial breast and/or ovarian cancer and 344 DNA samples from women with unselected ovarian cancer.

Results: Conducted investigation did not reveal any large mutations in the *BARD1* gene. As a side effect of the analysis, confirming the precision of our test, we detected 7 single-nucleotide substitutions, inducing a 30%–45% decrease in signal of single MLPA probe specific for either exon 8 or 10. Sequencing analysis led to the identification of 3 different sequence variants located within target sequence of respective MLPA probes. Two of them, c.1690C>T and c.1977A>G, were described earlier to induce aberrant splicing. The third, constituting missense mutation c.1972C>T, was reported as possibly pathogenic.

Conclusions: Concluding, although we cannot exclude the presence of large mutations in *BARD1*, our study indicates that such mutations do not contribute substantially to the risk of breast and/or ovarian cancer.

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P077

BARD1 Germline Mutations Lead to High Telomere Instability in Ovarian Cancer Patients

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Objectives: The tumour suppressor gene *BARD1* is a main binding partner of *BRCA1* and is required for both *BRCA1* stability and tumour suppressor functions. Differentially spliced isoforms of *BARD1*, lacking the N-terminal RING finger and *BRCA1*-interaction domain, are highly upregulated in breast cancer and other epithelial cell carcinomas. Additionally, our previous studies indicated that germline mutations of *BARD1* may predispose to breast and/or ovarian cancer.

Methods: *Study Population:* The study comprises 265 unselected ovarian cancer patients who were referred to the Department of Gynaecological Oncology of Medical University of Gdansk between 1995 and 2009.

Mutation Screening: Mutation analysis was done using a combination of high-resolution melt (HRM) and bi-directional sequencing.

Telomere PNA FISH Analysis: Lymphocytes from patients carrying *BARD1* mutations and from healthy donors were cultured according to standard procedure. Telomere FISH was carried out on metaphase chromosomes according to the manufacturer's protocol. Average number of each telomere aberration was calculated from at least 45 metaphases for each patient and 90 metaphases for healthy controls.

Results: Conducted analysis led to the identification of 4 possibly pathogenic mutations, including a novel mutation located in exon 5 (c.1361C>T, p.p.454L), an alteration located in exon 8 (c.1690C>T, p.Gln564X), and another in exon 10 (c.1977A>G, p.=). All of them result in incorrect splicing and overexpression of certain *BARD1* isoforms. Moreover, 1 of the patients was found to carry a recurrent mutation in exon 10 (c.1972C>T, p.R658Y) which was previously reported as cancer-related. Finally, telomere FISH experiments demonstrate that all of these alterations led to high telomere instability in patients' cultured lymphocytes when compared to healthy controls.

Conclusions: Our findings suggest that *BARD1* mutations may be regarded as cancer-risk alleles.

NON-*BRCA1/2* GENETIC FACTORS ASSOCIATED WITH CANCER RISK (CONTINUED)

P078

Rare Key Functional Domain Missense Substitutions in *MRE11A, RAD50,* and *NBN* Contribute to Breast Cancer Susceptibility: Results from a Breast Cancer Family Registry Case–Control Mutation-Screening Study

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Objectives: The Mre11A–Rad50–Nibrin (MRN) complex plays several critical roles related to repair of DNA double-strand breaks. Inherited mutations in the 3 components predispose to genetic instability disorders and the MRN genes have been implicated in breast cancer (BC) susceptibility, but the underlying data are not entirely convincing. We sought to estimate the frequencies and nature of MRN variants in 1311 women with early-onset BC and 1111 controls from the Breast Cancer Family Registry.

Methods: Using high-resolution melt curve analysis followed by Sanger sequencing, we mutation-screened the coding exons and proximal splice junction regions of the MRN genes in the 2422 subjects. Rare variants in the 3 genes were pooled using bioinformatics methods similar to those previously applied to *ATM, BRCA1*, *BRCA2*, and *CHEK2*, and then assessed by logistic regression.

Results: Because *ATM, BRCA1,* and *BRCA2* do not harbour pathogenic alleles (other than modest-risk SNPs) with minor allele frequencies >0.1% in Caucasian Americans, African Americans, or East Asians, we limited our MRN analyses to variants with allele frequencies of <0.1%. Combining protein truncating variants, likely spliceogenic variants, and key functional domain rare missense substitutions, we found significant evidence that the MRN genes are indeed intermediate-risk BC susceptibility genes (OR: 2.88, $p = 0.009$). Key domain missense substitutions were more frequent (24 vs. 12 observations) than the truncating variants and conferred a slightly higher OR (3.07 vs. 2.61) with a lower p value (0.029 vs. 0.14).

Conclusions: These data establish that *MRE11A, RAD50,* and *NBN* are intermediate-risk BC susceptibility genes. Their spectrum of pathogenic variants includes a relatively high proportion of missense substitutions. However, the data neither establish whether variants in each of the three genes are best evaluated under the same analysis model nor achieve clinically actionable classification of individual variants observed in this study.

P079

Exome Sequencing Analysis of a Rare Ovarian Cancer Family of French Canadian Descent Found Negative for *BRCA1* and *BRCA2* Mutations

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Family history of breast and/or ovarian cancer (OvCa) is a major risk factor for ovarian cancer and a history of OvCa in first-degree relatives doubles the risk. In French Canadian (FC) families with at least 2 cases of OvCa, inherited mutations in *BRCA1*/*2* contribute to 70% of cases. However, about 15% of families that have at least 1 OvCa case are *BRCA1*- and *BRCA2*-mutation negative, suggesting that other susceptibility genes may play a role. Using whole-exome sequencing, a rare FC family with 3 first-degree relatives with OvCa found negative for *BRCA1*/*2* mutations was investigated for other cancer susceptibility gene candidates.

Whole-exome sequencing analysis of blood DNA from 2 OvCa siblings that were *BRCA1*/*2*-negative was performed. Common rare variants between cases, associated with known genes were examined. Other variants were prioritized through functional consequence, prediction scores, and gene function focusing on cancer. Selected candidates were validated by Sanger sequencing and their frequencies were assessed by TaqMan genotyping assay in 450 OvCa cases.

Our analysis revealed 232 variants common between the two cases; 11 potential deleterious mutations, 13 splice-site, and 154 non-synonymous variants. Among these, variants in the Fanconi anemia I gene (*FANCI*) and the growth regulation by estrogen in breast cancer 1 gene (*GREB1*) were of interest. Genotyping experiments for the *FANCI* variant in OvCa cases have identified 4 new positive samples. Additional candidates involved in cell proliferation, gene expression, and DNA repair are currently being investigated.

Exome sequence analysis of a well-defined OvCa family generated candidates for further analyses as cancer susceptibility genes. Exome analyses of 6 additional families with at least 2 OvCa cases in first-degrees relatives are currently underway, and results will be compared. Identification of new OvCa susceptibility genes will improve the genetic testing and the management of women in *BRCA1*/*2*-negative families.

P080

Breast Cancer Gene Panel Testing Among High-Risk Individuals: A Single-Institution Experience

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Objective: Identifying individuals with hereditary predisposition to breast cancer has important implications. Next-generation sequencing (NGS) allows for rapid identification of multiple genes responsible for hereditary cancer risk and is being increasingly utilized in cancer genetics evaluation. This study presents the results of gene-panel testing for patients with significant history of breast cancer seen at Beaumont Health System.

Methods: Patients with suspected hereditary predisposition to breast cancer were evaluated at the Beaumont Cancer Genetics Program. Patients received genetic counselling and were informed of the implications and limitations of gene panel testing. The panels consisted of 6–26 genes associated with a risk for breast cancer, offered by 2 different laboratories in 5 different panel combinations. These genes were all evaluated using NGS and microarray technologies.

Results: Between November 2012 and January 2014, 57 patients underwent gene-panel testing, with 38 (66.7%) testing negative and 3 (5.3%) testing positive for a deleterious mutation (1 *PALB2*, 2 *PTEN*). Of the *PTEN* carriers, 1 did not meet testing criteria for Cowden syndrome. Sixteen patients were found to carry a variant, with 1 patient having 2 mutations (*BRCA1* and *BRCA2*), for a total of 17 variants (29.8%). Of the variants, 6 occurred in *BRCA1*/*2*, and 11 occurred in other genes (2 *CDH1,* 3 *CHEK2*, 2 *RAD50,* 1 *MUTYH,* 1 *ATM,* and 1 *CDKN2A*). Of the *CHEK2* variants, 2 were interpreted as deleterious by another laboratory.

Conclusion: This study demonstrates the finding of deleterious mutations in genes other than *BRCA1*/*2* that would likely not have been discovered by pedigree analysis alone. The limitation is that 29.8% of patients had a variant in a gene with unknown clinical implications. Further studies are needed to better define the broad mutational spectrum of high-risk families with breast cancer to optimize clinical management.

NON-*BRCA1/2* GENETIC FACTORS ASSOCIATED WITH CANCER RISK (CONTINUED)

P081

Identification of Hereditary Cancer Mutations in High-Risk Non-*BRCA* Breast Cancer Patients from Puerto Rico

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Introduction: Approximately 5%–10% of all breast cancers are caused by mutations in highly penetrant susceptibility genes such as *BRCA1* and *BRCA2*. Deleterious mutations in genes such as *PTEN*, *p53, CHEK2*, *ATM, NBS1, RAD50, BRIP1,* and *PALB2* also confer moderate-to-high risk of breast cancer, and together with *BRCA1* and *BRCA2*, they may explain up to 50% of breast cancer risk. Hispanics make up 16.3% of the population, and accounted for 56% of the national growth between 2000 and 2010. Yet these populations are often underrepresented in genetics studies. Our previous work has shown that the *BRCA* mutation spectrum underlying hereditary breast cancers in Puerto Rico is distinct from that of other Hispanic populations such as Mexican Americans.

Objective: Our objective was to identify the common germline mutations underlying non-*BRCA* breast cancer in the Hispanic population from Puerto Rico.

Methods: Our study recruited 16 breast cancer patients that met the clinical criteria for *BRCA* testing but had received a negative *BRCA1/2* result. Exons and intron–exon junctions of 52 tumour suppressor genes that have been previously implicated in hereditary cancer predisposition were captured using the Agilent SureSelect BROCA cancer risk panel. Massively parallel sequencing was performed on an Illumina MySeq platform.

Results: A total of 5846 variants were identified within the targeted gene regions. Each patient carried, on average, 72 coding variants, which included 41 synonymous variants and 31 non-synonymous variants. Work is underway to prioritize variants according to their likelihood of being a causative deleterious mutation.

Conclusions: Our findings may uncover genetic changes specific to this population that will allow for the development of custom-designed predisposition tests, taking into account natural differences in populations to maximize the identification of individuals at risk.

P082

The Status of Germline *TP53* Mutations in Breast Cancer Families and Cases of French Canadian Descent

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Objectives: Recently, we reported germline *TP53* mutations in 3.8% (2/52) of hereditary breast cancer (HBC) or hereditary breast and ovarian cancer (HBOC) families of French Canadian (FC) descent negative for pathogenic *BRCA1/2* mutations. Here, *TP53* mutation analysis was extended to 37 new HBC/HBOC families and FC invasive breast cancer cases not selected for family history of cancer. Independently, Montreal-based hereditary cancer clinics provided pedigrees of FC cancer families with *TP53* mutations to further describe the mutational spectrum of this gene in this population.

Methods: Group 1 subjects were cancer-affected index cases from 25 HBC and 12 HBOC families. Group 2 subjects were 1235 FC female breast cancer patients, of which 656 cases were diagnosed under age 50. Sanger sequencing was used to detect variants in protein coding exons 2–11 (group 1) or exons 5–9 (group 2) and adjacent splice sites of *TP53*.

Results: Six FC cancer families with *TP53* mutations were identified from hereditary cancer clinics; all carried missense mutations. Two families harboured identical mutations (Pro219Ser); review of the pedigrees suggested that those families are related. Mutation analysis of index cases from 37 HBC/HBOC families identified no mutation-positive families. Three *TP53* mutation-positive cases were identified among the 1235 breast cancer cases; all were diagnosed under age 50. One case harboured the same mutation (Arg282Trp) as a FC cancer family. The other 2 carried identical mutations (Cys229Arg). Eight different *TP53* mutations have thus far been identified in individuals or families of FC decent.

Conclusions: We estimate that the carrier frequency in HBC/HBOC families negative for pathogenic *BRCA1/2* mutations is approximately 2.2% (2/89). However, none of the research-identified *TP53* mutation-positive families or cases would have warranted an investigation of *TP53* in the clinic. Our findings would support the surveillance of *TP53* as part of the panel of breast cancer susceptibility genes.

PSYCHO-SOCIAL ISSUES

P083

Experiences of Women Affected by Familial Breast Cancer Receiving Breast Cancer Risks Derived from Common Genomic Variants

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Objectives: Genetic counselling provides information to patients about genetic risks of breast cancer. Currently most of the known high-risk genes are inherited in an autosomal dominant fashion—for example, germline mutations in *BRCA1* or *BRCA2*. However, the inheritance of common genomic variants and how they interact to increase an individual's risk of breast cancer is more complex. As testing for these variants becomes more widely available and is increasingly used by genetic health professionals, it is important to investigate how women understand and experience learning about this additional information.

Methods: We invited 40 women to receive their common genomic variant results via an appointment with a clinical geneticist or genetic counsellor at the Familial Cancer Clinic, Peter MacCallum Cancer Centre, Australia. Participants had been previously diagnosed with breast cancer and assessed as high-risk based on their family history of breast cancer. All women had undergone *BRCA1* and *BRCA2* screening, and no mutation was found. With consent, individuals were genotyped for 22 common variants from which breast cancer risks were calculated. An in-depth semi-structured interview was conducted ~1 week after the results appointment. Inductive qualitative analysis informed by grounded theory was used to identify themes.

Results: As of December 2013, 12 women who had received common genomic variant information were interviewed. They described their experiences of altruism and said they had gained an understanding of other genetic factors associated with their breast cancer diagnosis. Women's experiences and understanding were found to be influenced by their family history, breast cancer treatment outcomes, and prior uptake of preventive strategies (prophylactic mastectomy).

Conclusions: Results from this study can be used to identify more effective ways for genetic health professionals to communicate this complex genetic information and could inform policy on the introduction of common genomic variant testing in familial cancer clinics and genetic testing services.

P084

An Innovative Model of Care for Healthy *BRCA* Mutation Carriers: Evidence of Pre-implantation Genetic Diagnosis Uptake

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Objective: To evaluate the uptake of *in vitro* fertilization (IVF) and pre-implantation genetic diagnosis (PGD) between the years 2009 and 2012 in healthy *BRCA* mutation carriers of childbearing age attending a multidisciplinary clinic. PGD is typically offered to *BRCA* mutation carriers with the receipt of positive genetic results and may not consistently be followed-up unless pursued by the individual. The multidisciplinary clinic was initiated with the intent of providing a "one-stop shop" for recommended screening strategies, joint consultations with couples encountering difficulty in decision-making with issues pertaining to risk-reducing surgeries, hormone therapy, and reproductive options. This study evaluated the effectiveness of this innovative model of care and the response to recommended PGD.

Method: Participants include 163 *BRCA* mutation couples of childbearing age (25–35 years) who were previously informed of their option to undergo PGD and IVF procedure. The option and availability of PGD was reiterated to women of childbearing age attending the clinic during their first screening appointment.

Results: There are 1300 *BRCA* mutation carriers (men and women) recorded in the genetic data base of this medical facility, of which 163 are healthy *BRCA* mutation carriers between the ages 25 and 35. There are 130 healthy *BRCA* mutation carriers of childbearing age attending the clinic. Between the years 2009 and 2012, 33 couples elected to undergo PGD and IVF in our facility. Of the 33 couples, the *BRCA* carrier was the male counterpart in 9 couples. Of the remaining 24 couples, 22 couples attended the clinic. Only 2 couples were not known to the clinic.

Conclusion: The uptake of PGD and IVF is a complex process for healthy *BRCA* mutation carriers. Nevertheless, the numbers indicate that reiterating the option and availability of PGD in the context of attending a multidisciplinary clinic influences uptake in couples who are contemplating PGD and IVF.

PSYCHO-SOCIAL ISSUES (CONTINUED)

P085

The Role of Support from Family and Friends on Long-Term Cancer-Related Distress Following *BRCA1/2* Genetic Testing

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Objective: To examine the role of social support on long-term cancer-related distress following *BRCA1/2* test result disclosure among carriers and non-carriers.

Methods: A cohort of 91 *BRCA1/2* carrier and 135 non-carrier women were followed prospectively for 3 years after receiving their test results. Cancer-related distress was measured by the Impact of Event Scale. Social support (SS) was evaluated through 4 items measuring satisfaction with SS in general and satisfaction with SS to confide about genetic testing. Multivariate multi-level models were used to assess whether SS mediate or moderate the effect of mutation status on cancer-related distress.

Results: Cancer-related distress was higher among carriers than non-carriers at 1 year ($p = 0.01$), but this difference was no longer statistically significant 3 years ($p = 0.14$) after the test result disclosure. Carriers were less satisfied with their SS to confide about genetic testing compared to non-carriers ($p = 0.01$). SS moderated the effect of mutation status on cancer-related distress as carriers highly satisfied with their SS reported the same low levels of distress observed in non-carriers; carriers moderately or not satisfied with their SS reported high levels of cancer-related distress ($p < 0.01$).

Conclusions: For female *BRCA1/2* mutation carriers, social support seems to have a positive influence on the psychological adjustment process. The importance of social support needs to be stressed and possible ways to obtain support to confide about genetic testing issues addressed.

RISK ASSESSMENT AND GENETIC COUNSELLING ISSUES

P087

Population-Based Testing for *BRCA1* Mutations in the Bahamas

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Seven founder mutations in *BRCA1* and *BRCA2* predispose to breast and ovarian cancer in the Bahamas. We found a mutation in 27% of 214 unselected breast cancer patients in the Bahamas, including in 11% of unselected cases younger than age 40. We sought to evaluate the prevalence of mutations in unselected Bahamian women, regardless of age or cancer history. We tested 1300 women at large from the population, of whom 817 were identified through a workplace initiative at the largest resort in Paradise Island. We tested 817 women for mutations on a 3-day awareness campaign. Women who test positive will be offered post-test genetic counselling and referral for oophorectomy. The results of this testing initiative will be presented for the first time.

P088

Breast Cancer Risk Prediction and Risk Communication Practices: Evidence from a Canadian Survey

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Objectives: The aim of this paper is twofold: to explore the genetic counselling process for breast cancer and to compare family doctors, medical specialists, and genetic counsellors with regard to their genetic counselling practices.

Methods: This paper presents a cross-sectional study based on a Canadian medical doctors and genetic counsellors survey ($n = 176$) realized between July 2012 and March 2013. The data analysis included descriptive statistics, one-way ANOVA and *post hoc* tests.

Results: The genetic counselling process occurs within a chain of value-adding activities of 4 stages describing health professionals' clinical practices: 1) assessment, 2) investigation, 3) information, and 4) decision. Overall, results indicate that genetic counsellors stand out in the beginning of the process, while medical doctors are more active at the end of the process.

Conclusion: This paper presents a useful integrative framework to understand the current process of genetic counselling for breast cancer in Canada, and to shed light on how and where health professionals contribute to the process. The results also demonstrated the pertinence and unique role of genetic counsellors in the care provided to women at risk of familial breast cancer and offers a starting point for assessing clinical practices in genetic counselling.

RISK ASSESSMENT AND GENETIC COUNSELLING ISSUES (CONTINUED)

P089

A Cancer Risk Assessment Tool for *BRCA1* and *BRCA2* Carriers

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Background: Non-genetic risk factors such as age, family history, reproductive history (parity, age of menarche, breast feeding, oral contraceptives), and history of surgical prevention have been shown to vary cancer risk in women with a *BRCA1* or *BRCA2* mutation. It is important to provide women with an accurate projected lifetime risk of cancer so that they may choose between available prevention options. Currently, health care professionals rely on generalized computer models that often overestimate or underestimate patient risk, and to date there is no accepted application that can accurately calculate projected risks of a patient given their non-genetic risk factors.

Objectives: Our research question asks: What are the annual and lifetime risk of breast and ovarian cancer among women with a *BRCA1* or *BRCA2* mutation, and how are these risks modified with age, family history, parity, age of menarche, breast feeding, oral contraceptives, and oophorectomy?

Methods: Experimental data will be acquired from the *BRCA* carrier database at Women's College Research Institute. Annual and lifetime cancer risks will be estimated using prospective cohort data. Age-specific odds ratios of non-genetic risk factors will be estimated from baseline questionnaires using a case–control methodology. Exposure variables along with their interactions will be tested for significance using statistical methods. Covariates will be modelled using an unconditional multivariate logistic regression analysis. Models for *BRCA1*, *BRCA2*, breast cancer, and ovarian cancer will be selected based on maximizing the log likelihood statistic. Following the validation of the multivariate models, we will develop an open-source Web-based application that will allow users to input personal exposure variables. Application outputs will include breast and ovarian cancer penetrance curves based on a Cox proportional hazards model. Additional outputs will include penetrance curves had the patient undergone preventive measures. Users will be given the option to print or e-mail their results for their convenience.

P090

Quality Indicators of Genetic Assessment in the High-Risk Ontario Breast Screening Program

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Objectives: In 2011, the High-Risk Ontario Breast Screening Program (OBSP) expanded to include organized breast screening with annual mammography and breast MRI for women at high risk of developing breast cancer. Eligibility criteria included: ages 30–69 with i) *BRCA1*/*2* mutations, ii) 50% risk of *BRCA1*/*2* mutation, iii) ≥25% lifetime risk of breast cancer (by IBIS or BOADICEA), iv) prior chest irradiation. A standardized pathway with criteria for genetic assessment (GA) was implemented in 29 centres. The objective of this study was to evaluate quality indicators of GA.

Methods: Data were collected for women assessed July 2011 to June 2012. Variables included demographics, risk factors, breast cancer risk assessment, referral dates for GA, and testing information. Subjects consented to the use of their data for evaluation.

Results: Of 6835 women referred, 964 (14%) were known high-risk, and 5899 (86%) had GA. Of the 5899, 5201 completed GA and were studied. Median wait time for GA was 62 days (range: 3–145 days). 1852 Women (36%) had genetic counselling (GC) and genetic testing (GT); 3349 (64%) had GC only. Of GT results, 82% were available within 90 days. The median time to disclosure of GT results was 22 days (range: 9–42 days) after results were reported. Wait times were similar for women <50 vs. >50 years. The median wait time for GC was shortest for women with 50% mutation risk (16 days) and longest for women who did not meet high-risk criteria (65 days). Overall, 31% met the high-risk criteria.

Conclusions: After implementation of the OBSP high-risk program, many women received timely GA in Ontario. GC wait time ranged widely and varied by risk criteria. Possible explanations include variability in GC resources, and risk triage by GC centres. Less than one third of women referred met the high-risk criteria. Evaluation of the risk assessment process and referral criteria is needed.

P092

All in the Family: Barriers to Using Family History Questionnaires

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With increasing knowledge of the hereditary component to breast and ovarian cancer, the demand for cancer genetic counselling services has increased. The need to obtain family history information in a manner less reliant on a genetic counsellor has led to the development of the family history questionnaire (FHQ). Currently, FHQs are widely used and are an invaluable tool for assessing familial cancer risk and triaging patients for genetic counselling services. Despite its benefits, there remains a low return rate of mailed FHQs from newly referred patients, suggesting potential barriers to their use. A paucity of studies focusing on the barriers of both the design and implementation of FHQs exists. To address this, a total of 461 participants, 299 who completed the FHQ (responders) and 162 who did not (nonresponders), were surveyed regarding potential barriers to using the FHQ. Responders were more likely to state that their physicians discussed the reason for their appointment (<0.001). Interestingly, with respective rates of 51% and 56%, there was no significant difference in the proportion of responders and nonresponders who reported difficulty in completing the FHQ. However, our data demonstrate that responders and nonresponders both report large family size, lack of contact with relatives, and lack of knowledge of family history as major variables confounding completion of the FHQ. Of interest, more than 60% of the study population still preferred providing their family history of cancer in the form of a questionnaire, rather than by telephone or in person. Together, these data suggest that the barriers associated with the use of FHQs are not inherent to its design; therefore, the FHQ remains an effective tool for hereditary cancer clinics. These findings also shed light on the critical role that referring physicians and genetic counsellors play in reducing the size of the nonresponder population.

P093

Recent BRCAPRO Upgrades Significantly Improve Calibration

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Release 2.0-8 of the BayesMendel genetic risk assessment package contains an updated BRCAPRO model, including new estimates for contralateral breast cancer (CBC) penetrance, consideration of mixed-pedigree ethnicities, and consideration of mastectomies.

Objectives: In this work, we report the results of a validation of the new BRCAPRO using Cancer Genomics Network (CGN) pedigrees, comparing it with the previous release (2.0-7). We also describe the methodology followed to obtain the CBC penetrance curves from the literature.

Methods: From a statistical modelling standpoint, the penetrance curves have been evaluated for carriers of a mutation in either of the *BRCA* genes by parametric modelling of the survival data published in *J Clin Oncol* 2009;27(35).

A preliminary analysis of the SEER 9 database for patients experiencing up to 3 relapses of invasive CBC, has allowed for an evaluation of penetrance for non-carriers by deconvolution.

Results: In general, the two versions of BRCAPRO have similar power in discriminating *BRCA1* and *BRCA2* carriers from non-carriers and a similar MSE, but the calibration significantly improves to a 0.98 observed vs. expected ratio in version 2.0-8 compared to 0.89 in version 2.0-7. For families reporting a diagnosis of CBC, the Net Reclassification Index (NRI) of BRCAPRO 2.0-8 at a 0.05 threshold is 0.08.

Conclusion: We observe a general improvement in BRCAPRO with a reduction of the previously overestimated carrier risk both overall and in families with CBC; this improvement is achieved with minimal additional carrier misclassification compared to the previous version.

RISK ASSESSMENT AND GENETIC COUNSELLING ISSUES (CONTINUED)

P094

Prospective Evaluation of the Potential to Reduce Breast Cancer Risk Through Lifestyle Modifications in *BRCA*-Mutation Carriers

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Background: In recent years, there has been increased penetrance of *BRCA*-mutations which may be due to lifestyle influences. There is a need to identify approaches to reduce penetrance of *BRCA* mutations. Understanding how modifiable lifestyle factors affect cancer risk in *BRCA* mutation carriers may have implications for risk reduction in this group. At the molecular level, oxidative stress and genomic instability are early events in cancer development, and these processes may be considered surrogate markers of cancer risk. *BRCA* mutation carriers are more susceptible to these pro-carcinogenic processes than non-carriers. There is some evidence that obesity and physical inactivity promote oxidative stress, though this has not been investigated in *BRCA* mutation carriers.

Objective: The aim of this pilot study was to prospectively examine the effect of physical activity and lifestyle factors on oxidative stress profiles in a cohort of unaffected *BRCA* mutation carriers.

Methods: Participants ($n = 68$) were recruited from breast cancer family-risk clinics and cancer genetics clinics. Body composition (BMI, waist-circumference, adiposity), metabolic profiles, and physical activity (Minnesota Leisure-Time Physical Activity Questionnaire) were measured for each participant. Serum levels of the oxidative stress markers 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-DG) and 4-hydroxynonenal (4-HNE) were measured in a subset of participants ($n = 16$) by ELISA.

Results: Participants demonstrated poor adherence to physical activity guidelines, with 93% of our cohort not reaching recommended physical activity levels. Analysis of body composition in this cohort revealed that the majority were overweight (37%) or obese, (34%) with 72% exhibiting abdominal obesity. Correlation of serum levels of oxidative stress markers with physical activity and lifestyle factors revealed a novel inverse association between physical activity levels and serum markers of oxidative DNA damage (8-oxo-DG) and lipid-peroxidation (4-HNE). These associations trended towards statistical significance ($p = 0.08$ and 0.07 respectively).

Conclusion: This pilot work has provided compelling evidence that, in this cohort of *BRCA* mutation carriers, unhealthy lifestyle patterns are prevalent. In addition, these results suggest that the potential may exist to modify pro-carcinogenic processes in this cohort through physical-activity modifications, and this is currently under further investigation in our laboratory.

P095

An Investigation into the Presence of Modifiable Breast Cancer Risk Factors in *BRCA* Mutation Carriers

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Background: Sporadic breast cancer (BC) risk is modifiable through alterations in lifestyle-related risk factors including body weight and associated dysmetabolism. In recent years there has been increased penetrance of *BRCA*-related BC suggesting that lifestyle behaviours may modify risk in this genetic cohort. Maintenance of a healthy body weight throughout early adulthood is suggested to reduce *BRCA*-related BC risk; however, there is a lack of objective data. We describe baseline anthropometric and metabolic findings from a prospective longitudinal study which will objectively measure the association between modifiable risk factors and *BRCA*-related BC risk.

Methods: Women with genetically identified *BRCA1* and *BRCA2* mutations, with no history of cancer were recruited from family risk assessment and genetics clinics at St. James's Hospital, Dublin, Ireland. Anthropometric measurements including body mass index (BMI) and waist circumference (WC) were completed. Fasting insulin, glucose, and lipid profile samples were drawn for metabolic screening. Insulin resistance was estimated using the homeostatic model assessment index (HOMA-IR). Participants were also asked for their opinion on whether obesity was a risk factor for BC.

Results: To date, 73 participants have enrolled [mean (SD) age: 41.8 (9.3) years]. The average BMI score was 28.6 (6.3) kg/m^2, with the majority of participants either overweight ($n = 28$) or obese ($n = 25$). Central obesity was a prominent characteristic, with a mean WC of 88.8 (14.3) cm and more than 75% of participants ($n = 55$) centrally obese (WC > 80 cm). Clinically defined metabolic syndrome was present in 16 participants, and 20 participants were insulin-resistant. The majority ($n = 60$) thought that obesity was a risk factor for BC.

Conclusions: Increased body weight and central obesity is problematic in this cohort despite the burden of increased disease risk and the knowledge that obesity may modify risk. While current results are limited to a descriptive analysis, the prospective study will examine the association between identified risk factors and *BRCA*-related BC occurrence.

P097

The Ontario Breast Screening High-Risk Program: The Eastern Ontario Regional Genetics Program Experience in the First Year (2011–2012)

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The Ontario Breast Screening Program (OBSP) is a province-wide organized breast screening program managed by Cancer Care Ontario, providing high-quality breast cancer screening for women aged 50–74 years since 1990. In 2011, OBSP expanded to screening of women at high risk (HR) for breast cancer (OBSP-HR), with annual breast MRI in addition to mammography. HR women include 1) carriers of a deleterious gene mutation, 2) first-degree relatives of a mutation carrier who have declined genetic testing, 3) those with a >25% lifetime risk of breast cancer (BC) as assessed by a genetics clinic using either IBIS or BOADICEA risk models, 4) those who have received chest radiation before age 30 and at least 8 years previously. Genetic centres from 14 Local Health Integration Networks (LHINs) play a critical role in the OBSP-HR by providing genetic counselling (GC), genetic risk assessment (GRA), and genetic testing (GT) to women who may be at HR for BC. The GRA determines eligibility for breast screening through OBSP-HR. The Eastern Ontario Regional Genetics Program at CHEO is part of Champlain LHIN. In the first year of the OBSP-HR program, 6863 women in Ontario were registered (14% with established HR, 86% referred for GRA). Of referrals for GRA, 57% completed GC, 31% completed GT, and 35.5% of evaluated women were found eligible for HR screening. Of eligible women, 97% were screened, with a 27.7% abnormal call rate. In Ontario, 35 women were found to have BC. In Champlain LHIN, 920 women were registered, 661 received GRA, and 36% of those receiving GRA were determined eligible for HR screening. We will compare the characteristics (age, risk criteria, BC history) of women screened from Champlain LHIN to provincial data. We will also compare times from referral to GC, GT, and screening.

P098

Genetic Anticipation in Breast Cancer Associated with the *BRCA* Mutation

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Objectives: Data on genetic anticipation in breast cancer are sparse. We sought to determine if there is a change in the age at diagnosis of breast cancer between mothers and daughters with a known *BRCA* mutation.

Methods: A review of all the carriers of the *BRCA* mutation diagnosed with breast cancer at our Genetics Institute yielded 80 women who could be paired with a mother with breast cancer who is either a *BRCA* mutation carrier or clinically likely to be according to pedigree analysis. Age at diagnosis, type of mutation, year of birth, and ethnicity were recorded. Paired *t*-test was used to analyze differences in age of cancer onset between groups and subgroups.

Results: Mean age at diagnosis of breast cancer was 51 years (range: 22–88 years) in the mothers and 44 years (range: 24–75 years) in the daughters. The difference (6.8 years) was statistically significant ($p < 0.001$). A significant difference in age at onset was also found on separate analysis of pairs in which the daughter was born after 1950 or 1960. These findings were consistent regardless of type of *BRCA* mutation, ethnic origin, or mothers' year of birth. By contrast, there was no significant difference in mean age at diagnosis between pairs in which the mother was diagnosed before age 50 years: 41.5 years (mothers) versus 42.4 years (daughters).

Conclusion: Daughters who carry the *BRCA* mutation are diagnosed with breast cancer at an earlier age than their carrier mothers. The only exception to this pattern are pairs in which the mother was diagnosed before age 50 years. In the future, specific breast screening guidelines may be developed for the different subpopulations of *BRCA* mutation carriers.

RISK ASSESSMENT AND GENETIC COUNSELLING ISSUES (CONTINUED)

P099

Trends in *BRCA*-Predictive Testing in Northern Ireland

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Objectives: Since 1995, 624 women in Northern Ireland (NI) have been shown to carry *BRCA1* or *BRCA2* mutation. A positive test in a woman with breast or ovarian cancer provides her unaffected relatives the opportunity of predictive genetic testing. This study aimed to examine trends in predictive genetic testing between 1995 and 2013.

Method: Index cases were identified by retrieving information from the regional Genetics Department database, and the NI Clinical Oncology Information System provided details on those women who had breast or ovarian malignancy. Testing of unaffected women is routinely offered only to women within known *BRCA* families. Case notes were retrieved from the Family History Breast Clinic to obtain details of patient management in women with positive predictive testing.

Results: In 2000, only 1 of 14 women had predictive testing, but there has been a steady increase in predictive testing, and in 2013, 53% of tests were performed in unaffected women. There was no predictive testing in women under 30 years prior to 2002, but in 2013, 46% of predictive tests were in women under 30 years old. Since 2008, 121 women who had positive predictive testing attended the Belfast City Hospital. In women aged 40–59 years, 55% proceeded with breast risk-reducing surgery (BRRS) with or without bilateral salpingo-oophorectomy. This contrasts with the women under 30, only 15% of whom have proceeded with BRRS. These women will not be eligible for the high-risk breast screening programme until they are 30.

Conclusion: This study shows that more women in *BRCA* families in NI are having genetic testing in their 20s. The impact of a positive test result may be different in these younger women who have to wait for breast screening to start and are less likely to proceed with breast risk-reducing surgery.

P100

Breast Cancer Risk in Women Who Test Negative for a Familial *BRCA1* or *BRCA2* Gene Mutation: The Eastern Ontario Regional Genetics Program Experience

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Once a mutation in the *BRCA1* or *BRCA2* gene is identified in a family, at-risk relatives can choose to be tested. Women who are found to carry the familial mutation are counselled about their increased cancer risks. Women who are not found to carry the familial mutation are sometimes reassured that they are not at increased risk for breast cancer. Literature concerning the risk of breast cancer in women testing negative for a familial mutation continues to evolve, with some studies showing increased risk in the setting of a strong breast cancer history in close relatives.

In our genetics clinic, since the initiation of the Ontario Breast Screening high risk program in July 2011, most women who tested negative for a familial mutation have had a risk assessment using either IBIS or BOADICEA risk assessment tools. We will look at all of the women who tested negative for a familial mutation between July 2011 and December 2013 ($n = 130$) and describe the percentage of women whose lifetime risk for breast cancer is increased above general population risk and those eligible for high-risk breast screening.

In our initial cohort of 50 unaffected women, 50% were first-degree relatives of a mutation carrier. The mean age was 44.6 years (range: 19–86 years). The mean residual lifetime risk for breast cancer was 11.2% (range: 1.2%–29.8%), compared to 8.0% in the general population. The mean relative risk for breast cancer was 1.3 (range: 0.5–2.9). One patient was still found to be eligible for high-risk screening (≥25% lifetime risk). All 3 women with lifetime risk above 20% were below age 27. None of the women in the initial cohort had a personal history of proliferative breast disease. Updated data pertaining to the entire population of mutation-negative women ($n = 130$) will be presented.

P101

Population Screening for *BRCA* Mutations: What Is the Optimal Screening Program?

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Population screening for the *BRCA1/2* mutations common in Ashkenazi Jews (AJ) fulfills WHO screening criteria (high carrier rate, high sensitivity, and proven effective prevention). Clinical *BRCA* testing is offered to high-risk women through physician referral, with pre- and post-test genetic counselling. A *BRCA* screening program would target the general population and require alternate paradigms for referral and genetic counselling.

We aim to examine models for *BRCA* screening in Israel and to compare their efficacy in terms of uptake, carrier rates, satisfaction, knowledge, and psychological effects.

Screening is offered to healthy AJ, age >25. Enrollment is by self-referral (posters and brochures) or a study recruiter at mammography and executive screening clinics. Pre-testing participants receive written information and a family history questionnaire for risk assessment. Post-test counselling is provided to carriers and participants with significant family history. Psychosocial aspects are examined using questionnaires at 2 weeks (Q1) and 6 months (Q2).

Of 1727 individuals screened so far (64% uptake), 54% were recruiter-enrolled, and 46%, self-referred. Self-referred participants were significantly younger ($p < 0.001$) and had more suggestive family history of cancer ($p < 0.001$). At Q1, more than 90% of participants reported high/very-high satisfaction, with self-referred significantly more satisfied ($p < 0.001$). Mean knowledge score was 70%. Stress was reported by 1.5% after testing, but resolved in all non-carriers by Q2. Carrier rate was 1.9%, higher (2.6%) among self-referred. Of carriers, 44% had no suggestive family history and would not have been candidates for clinical testing.

There is a reasonably high acceptance rate for *BRCA* screening in AJ. In this study, self-referral was associated with higher carrier rates, younger age, more significant family history, and higher satisfaction. Lack of pre-test counselling is unorthodox, but our results suggest high levels of satisfaction, knowledge, and coping with this process. These results will inform future implementation of genetic advances into the public health arena.

P102

The Prevalence of *BRCA* Mutations Among Patients with Triple-Negative Breast Cancer

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Background: Several studies have addressed that breast cancers associated with *BRCA1* mutations are likely to be triple-negative, and triple-negative breast cancer is associated with *BRCA1/2* mutation.

Several studies have reported that 9%–14% of women with triple-negative breast cancer carry *BRCA1* mutation and 2%–4% of women with triple-negative breast cancer carry *BRCA2* mutation. However, there are limited data regarding the prevalence of *BRCA1/2* mutation among Japanese breast cancer patients.

Methods: We retrospectively investigated 154 women who were diagnosed with triple-negative breast cancer and underwent surgery between January 2011 and July 2013 at St. Luke's International Hospital. We analyzed the rate of implementation of genetic counselling and genetic testing, and the rate of gene mutations. In addition, we conducted a detailed search of the family history.

Results: In 239 women who were diagnosed with breast cancer and received genetic counselling, 123 patients received a genetic test (51.5%). Among the 123 patients who received genetic testing, 19 patients (15.4%) with *BRCA1/2* mutations were identified.

Among 154 patients with triple-negative breast cancer, 35 patients (23.2%) received genetic counselling. Among 24 patients (29.2%) who received genetic testing, 6 deleterious *BRCA1* mutations and 1 *BRCA2* mutation were identified. In 66 patients (42.9%) under 50 years of age, 25 patients (37.9%) received genetic counselling, and 18 patients (27.2%) received genetic testing. Six patients (33.3%) proved to be *BRCA1/2* mutation carriers. Two patients who were in their late thirties and late forties with *BRCA1/2* mutations had no family history.

Conclusions: Japanese triple-negative breast cancer patients may have higher chance for *BRCA* mutations—more than we expected. We are conducting a nationwide survey for the prevalence of *BRCA* mutations in triple-negative breast cancer in Japan.

RISK ASSESSMENT AND GENETIC COUNSELLING ISSUES (CONTINUED)

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All Ovarian Cancer Cases Should Be Fully Tested for *BRCA1/2*

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Background: In Norway, 6% of incident ovarian cancers carry a deleterious *BRCA1/2* mutation (Kotar *et al. J Med Gen* 2004), and the 2 most frequent mutations account for one third of the cases (Borg *et al. Dis Markers* 1999). From a start of applying testing for locally frequent mutations only, we offered complete genetic testing of *BRCA1* and *BRCA2* to all women in breast–ovarian cancer kindreds (FBOC) when capacity became available. As of 2011, however, our medical files indicated that a number of ovarian cancer FBOC cases were not fully tested.

Methods: All ovarian cancer cases referred to us since 1989 have been invited to mutation testing when becoming available, and blood samples were stored. All ovarian cancer patients who had consented to testing, but who had not been fully tested, were identified and tested according to informed consent and IRB approval.

Results: The 191 patients identified were subjected to Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). Twelve (6.3%) were found to have deleterious mutations, 7 in *BRCA1* and 5 in *BRCA2*. Fifteen variants of uncertain clinical significance (VUS) were identified. Mean age of diagnosis was 54.6 years (range: 41–77 years) including both genes; it was 53.3 years (range: 41–77 years) and 56.4 years (range: 42–66 years) for *BRCA1* and *BRCA2* respectively. We have offered predictive testing to 53 relatives, and 8 have tested positive to date.

Discussion: The finding of 6.3% mutation carriers came in addition to the previous findings when testing for locally frequent mutations only in FBOC. We have previously reported that, in a series of incident ovarian cancers, 2 of 3 *BRCA* mutation carriers did not fulfill our family history-based criteria for genetic testing (Møller *et al. Eur J Cancer* 2007). Together, our previous and present results indicate that all ovarian cancer cases should be offered full testing for *BRCA1/2* mutations.

P104

Are There Differences in Predictive Genetic Testing Uptake Among Families with Hereditary Breast/Ovarian Cancer or with Lynch Syndrome?

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Objectives: Several studies have been carried out on predictive genetic testing uptake, particularly in hereditary breast/ovarian cancer (HBOC) or Lynch syndrome. However, no data directly comparing these two cancer predisposition syndromes are available. This study aims to evaluate the uptake of predictive testing among relatives of index cases concerned by HBOC or Lynch syndrome diagnosed in a single oncogenetic unit, and to determine demographic and clinical predictors associated with this uptake.

Methods: Pedigrees of 115 consecutive *BRCA1/2* (n = 82) and Lynch syndrome (n = 33) families managed in the Oncogenetics and Cancer Prevention Unit at Geneva University Hospitals between 1995 and 2012 were extensively revised. Demographic and clinical characteristics were collected for all index cases as well as for 1st-, 2nd-, and 3rd-degree relatives alive and older than 18 years in 2013 (n = 1589). Predictive analyses performed before December 31, 2013, were recorded. Average follow-up period was 30 months.

Results: Average number of relatives/family did not differ between the two cancer predisposition syndromes. Most predictive analyses (174/219, 79.5%) were performed within 12 months after index cases had completed genetic screening. More analyses were performed among Lynch syndrome than among HBOC families (22.4% vs. 12.9%, $p < 0.0001$). For both syndromes, factors statistically significantly associated with the decision to perform predictive testing were close relationship with index cases and being affected by cancer ($p < 0.003$). Having offspring and female sex were associated with predictive testing only among *BRCA1/2* families ($p < 0.0001$). Comparison of characteristics of relatives performing predictive analysis showed a lower uptake among men concerned by HBOC compared to Lynch syndrome (6.4% vs. 22.7%, $p < 0.0001$).

Conclusions: After identification of pathogenic variants, it is essential to motivate and to help index cases to improve familial communication, particularly to male relatives and distant family members. An active follow-up may allow the uptake of predictive analysis to be improved.

P106

Evaluating the Performance of BOADICEA and Manchester Scoring System for Predicting the Risk of Having a *BRCA* Mutation in an Asian Breast Cancer Cohort

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Objectives: To date, risk assessment for *BRCA1* and *BRCA2* is largely based on age of onset and family history of breast or ovarian cancer. Given that there is a lower population risk to breast cancer and poorer reporting of cancer history among Asians, and that penetrance may be different in Asians, it is unclear whether risk prediction models accurately predict risk of carrying *BRCA* mutations in Asians. In this study, we have evaluated the accuracy of the statistical polygenic model BOADICEA and the empirical Manchester Scoring (MS) method in a cohort of Asian breast cancer cases.

Methods: From 2003 to 2012, 1692 incident and prevalent breast cancer cases were recruited to the Malaysian Breast Cancer Genetic Study, of whom 665 with early age of onset and/or family history of breast cancer were screened for germline mutations in *BRCA1* and *BRCA2*. We calculated the *BRCA1* and *BRCA2* carrier probabilities using BOADICEA (version 2.0) and the modified Manchester Scoring (MMS) method. For 577 individuals where pathologic data were available, we determined the added value of ER-negativity on the models.

Results: At 10% carrier thresholds, the area under curve (AUC) was similar for BOADICEA and MMS, and higher for *BRCA1* compared to *BRCA2* (0.73 and 0.73 for *BRCA1* and 0.66 and 0.64 for *BRCA2* in BOADICEA and MMS respectively). Addition of ER status improved the AUC for *BRCA1*, from 0.73 to 0.81 in BOADICEA and to 0.79 in MMS. Addition of other pathologic features (PR and HER2) did not improved *BRCA1* accuracy (AUC: 0.82). As for *BRCA2*, addition of pathologic features did not change the AUC for *BRCA2* in either model.

Conclusion: Our findings indicate that BOADICEA and MMS performance was similar to that reported in studies from Caucasian populations.

RISK ASSESSMENT AND GENETIC COUNSELLING ISSUES (CONTINUED)

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Breast Cancer Risk Associated with Factors Related to Menstruation and Menopause According to Nonsense MutationLocation in the French National *BRCA1/2* Carrier Cohort, GENEPSO

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Introduction: Mutations in *BRCA1* and *BRCA2* confer a high risk of breast cancer (BC), but the magnitude of this risk seems to vary according to various factors. In particular, there are data to support the hypothesis of allelic risk heterogeneity.

Methods: We assessed variation in BC risk according to factors related to menstruation and menopause by location of mutation in *BRCA1* and *BRCA2* in 990 women by using a weighted Cox regression model.

Results: Our results confirm the existence of a negative association between age at menarche and BC risk among *BRCA1* and *BRCA2* mutation carriers [>12 vs. <12 hazard ratio (HR): 0.69; 95% confidence interval (CI): 0.49 to 0.97] and suggest an association between short menstrual cycles and BC risk (20–24 days vs. 24–31 days HR: 1.73; 95% CI: 1.12 to 2.68) for *BRCA1* mutation carriers only. An increased risk of BC associated with a natural menopause ($p = 0.01$) and particularly at a late age was found for *BRCA1* mutation carriers (natural menopause >50 years vs. pre-menopause HR: 4.84; 95% CI: 1.63 to 14.4). These associations seem vary according to the location of mutation: that is, they were observed only for *BRCA1* mutation located between codons 373 and 1162 (for example, post-menopause vs. pre-menopause HR: 2.15; 95% CI: 1.30 to 3.55; interaction $p = 0.04$). Whereas no association with menopausal status was found for all women carrying a mutation in *BRCA2*, when location of the mutation is taken into account, an association was found for mutation located between codons 2546 and 2968 (HR: 4.01; 95% CI: 1.21 to 13.2; interaction $p = 0.01$).

Conclusions: Our findings show that environmental/lifestyle factors may have a differential effect on the BC risk according to the location of the *BRCA1/2* mutation. These results should be verified in larger population (for example, IBCCS for International *BRCA1/2* Carrier Cohort Study). These findings, if validated, could be incorporated into risk prediction models and help in the clinical management of *BRCA1* and *BRCA2* mutation carriers.

P108

Group Counselling Sessions for Unaffected Women with a Family History of Breast and/or Ovarian Cancer as an Effective Method of Identifying Individuals Eligible for Genetic Testing

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Since May 2011, in response to increasing demands for genetic counselling and risk assessments, the Inherited Cancer Program of the Children's Hospital of Eastern Ontario has been delivering group information sessions to a proportion of our patients. Women are considered eligible for group counselling when they have a significant family history of breast and/or ovarian cancer, but have not been personally diagnosed, nor is there a known family history of a mutation in a cancer susceptibility gene. This process has allowed us to provide cancer risk assessments to a large volume of patients, as well as identify individuals who are at a significant risk to carry a *BRCA1* or *BRCA2* mutation. While only a minority of group patients are subsequently offered testing, these sessions have encouraged the referral for genetic counselling of other family members who are eligible for genetic testing due to their own cancer diagnoses. Our objective is to review the utility of our group counselling sessions in identifying women at high risk for developing breast cancer when it leads to genetic testing of unaffected patients or their affected relatives. Between May 2011 and December 2013, a total of 946 individuals were assessed through group information sessions. These sessions led to the identification of 27 women from families without known *BRCA1/2* mutations who were subsequently offered genetic testing. We conclude that group counselling sessions for hereditary breast and ovarian cancer have been a successful means of identifying women at increased risk of carrying *BRCA1* and *BRCA2* mutations by way of unaffected family members. This has allowed us to provide a more accurate risk assessment to a greater number of women at increased risk for breast and ovarian cancer.

P109

Use of Cancer Genetic Services Among Young Breast Cancer Survivors and High-Risk Relatives

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Objectives: Young breast cancer survivors (YBCSS) and their relatives are at risk for hereditary disease and need referrals for cancer genetic services (counselling and/or testing). The study presents baseline use of cancer genetic services among YBCSS and their high-risk female relatives that was examined for a randomized efficacy trial regarding awareness of breast cancer genetics.

Methods: The Michigan cancer registry was used to identify a random sample of 3000 YBCSS, diagnosed at 25–45 years, and stratified by race (black vs. other). Consent forms and baseline surveys were mailed to YBCSS. YBCS responses helped identify up to 2 high-risk relatives without cancer. Overall, 862 high-risk relatives were invited to participate.

Results: In total, 883 YBCSS (average of 11 ± 4 years post-diagnosis) and 441 relatives returned a baseline survey. Approximately 30% of families were black and <13% were of Ashkenazi, Dutch, or Swedish backgrounds. Although most participants had health insurance and a usual source of care, only 33% YBCSS reported genetic counselling, and 29% had genetic testing. Only 3% of high-risk relatives had genetic testing. The rate of finding a *BRCA1/2* mutation was 18% white/other and 15% black; 10% and 20% respectively had a variant of unknown significance. Black YBCSS were less likely to report receiving a recommendation for genetic testing. Oncologists and family members were common sources of information about cancer genetics. Reasons for testing included benefiting the family, knowing about future health risks, and following provider recommendations. Lack of provider recommendation, lack of insurance coverage, and out-of-pocket expenses were common reasons for not getting testing.

Conclusions: Among YBCSS and high-risk relatives, the proportion of *BRCA1/2* mutations is high and almost identical for black and white/other. Black women were less likely to use cancer genetic services. There is lack of awareness of cancer genetics among relatives.

RISK ASSESSMENT AND GENETIC COUNSELLING ISSUES (CONTINUED)

P110

Comparison of *BRCA1/2* Mutation Probabilities and Outcomes Using BOADICEA versus Ontario Ministry of Health Eligibility Criteria

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Background: In 2000, the Ontario Ministry of Health and Long-Term Care (MOHLTC) established 13 eligibility criteria for *BRCA1/2* genetic testing for individuals/families with a significant family history of breast/ovarian cancer. Since then, new evidence has emerged to suggest that some of these criteria are weak (that is, <10% mutation detection), additional criteria are missing (that is, triple-negative status, prostate/pancreatic cancer), and online risk assessment tools are now available (that is, BOADICEA).

Objectives: Recognizing that Cancer Care Ontario is currently seeking to review the provincial criteria, with the primary objective of making them evidence-based, we wanted to compare the *BRCA1/2* mutation detection rates between the Ontario MOHLTC criteria and BOADICEA in our patient population.

Methods: 246 Charts were reviewed on women (age 29–69) who participated in *BRCA1/2* testing between July 2011 and November 2013 and had a BOADICEA risk assessment completed as part of their eligibility assessment for breast MRI through the Ontario Breast Screening (OBSP) high-risk program. This analysis included women who were offered *BRCA1/2* testing based on only 6 of the 13 MOHLTC eligibility criteria, because there were too few patients tested in this time frame who met other criteria.

Results: Overall, a deleterious mutation was found in 14/246 patients (5.7%); however, the mutation detection rate was >10% in only 2 of the eligibility categories (groups 6 and 7). Of those patients in whom a mutation was identified, the BOADICEA mutation likelihood was >10% in 10/14 patients (71%).

Conclusion: These findings further demonstrate that some of the MOHLTC criteria are weak at identifying patients at high risk, thus leading to over-screening in Ontario. A further review of *BRCA1/2* testing outcomes in other regions of Ontario is warranted, including a comparison across eligibility criteria using the BOADICEA risk assessment tool to determine its utility in this population.

P111

Online Use of Breast Cancer Risk Prediction Tools: Views of Women with a Family History of Breast Cancer

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Background: Family history is the main risk factor for breast cancer. Clinical risk prediction tools such as BOADICEA and BRCAPRO are increasingly used in cancer genetics. Online access to these tools would allow women to self-assess their risk of breast cancer. However, the psychosocial, medical, and ethical implications of such an application of these tools are yet to be explored.

Objective: To explore the views of women with a family history of breast cancer regarding an online use of clinical tools for risk estimation.

Method: Six focus groups were conducted with 34 women with a family history of breast cancer, recruited in genetic counselling.

Results: The perceived benefits include the possibility of raising public awareness about the importance of knowing one's own family history of breast cancer. Another is the ability to stay informed of one's own risk level based on the advancement of scientific knowledge. The perceived drawbacks include potential misinterpretation of results, lack of medical care and an increase in anxiety. The pertinence of using these tools online has also been questioned.

Conclusion: Several strategies would maximize the online implementation of clinical tools for predicting the risk of breast cancer. For example, these tools should be easy to use, provide clear results, and be accompanied by medical monitoring and individualized psychosocial support. In the context of the growing field of personalized medicine, more studies are needed to explore the ethical and organizational implications of using these tools online.

P112

Devrait-on en faire plus et élargir les indications de tests génétiques pour les patientes atteintes de cancer du sein et leurs familles?

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Introduction : Depuis l'identification des gènes *BRCA1–2,* plusieurs outils de calculs de risque ont été élaborés afin d'aider les cliniciens à déterminer la probabilité de trouver une mutation dans l'un de ces gènes. La réduction du coût des tests génétiques rend légitime d'évaluer la pertinence d'élargir les critères de sélection afin d'augmenter les taux de détection. Les recommandations de suivi et de prise en charge chirurgicale pourraient être adaptées dans un plus grand nombre de familles à risque.

Objectifs : En offrant des analyses génétiques pour les gènes *BRCA1–2* aux familles ne répondant pas aux critères de tests, selon les modèles couramment utilisés (BRCAPRO, Manchester), nous souhaitions déterminer si la limitation des analyses aux familles présentant un spectre tumoral fort suggestif pourrait mener à un sous diagnostic.

Méthodes : Étant donné l'ascendance majoritairement canadienne-française de notre population de patients et la possibilité d'offrir un panel de mutations fréquentes des gènes *BRCA1*–2 chez les personnes de cette origine, nous avons élargi nos critères de sélection. Parmi les familles où une mutation a été identifiée, nous avons revue avec attention l'histoire personnelle et familiale afin de s'assurer de la justesse des informations recueillies et avons confirmé certains diagnostics.

Résultats : Des mutations dans les gènes *BRCA1–2* ont été identifiées dans plusieurs familles ne répondant pas aux critères de tests. Dans ces familles, le spectre tumoral habituellement associé aux mutations dans ces gènes est peu présent. Des critères de sélection plus stricts n'auraient pas permis d'identifier des mutations chez ces familles.

Conclusion : L'élargissement de l'offre de tests permet d'identifier des mutations dans des familles où le syndrome de cancers du sein et des ovaires était peu suspecté au départ. La répercussion sur les coûts de santé et l'impact pour les familles est indéniable. Il est fort probable que dans un futur proche, l'approche multigène par séquençage de nouvelle génération et analyse de l'exome permettra de mettre en évidence un spectre phénotypique nouveau et possiblement atténué, associé aux syndromes génétiques déjà décrits.

P113

Quelle approche diagnostique pour les patients testés *BRCA* négatif? Devrait-on changer le fusil d'épaule?

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Introduction : Les familles à risque élevé de cancers du sein et de l'ovaire, dont les investigations pour les gènes *BRCA1–2* se sont révélées négatives, nous questionnent sur leur risque réel. Ces familles représentent une grande proportion des familles investiguées. Les recommandations de suivi pourraient être nuancées dans de nombreux cas.

Objectifs : À travers l'évaluation de notre approche clinique, nous voulions identifier dans quelles proportions une analyse de *BRCA1–2* peut être considérée rassurante.

Méthodes : De juillet 2011 à décembre 2013, nous avons considéré systématiquement, pour tous nos patients, un large éventail de causes syndromiques prédisposant au cancer du sein. L'anamnèse complète retraçait l'histoire personnelle néonatale, développementale, les facteurs de risque et événements sur la santé, jusqu'au diagnostic du cancer, ainsi que l'histoire familiale. L'examen clinique ciblé était réalisé lorsque l'anamnèse pouvait orienter vers une condition précise comportant un risque de cancer du sein.

Résultats : Nous avons identifié par cette approche, deux familles avec syndrome de Cowden, deux familles avec mutation dans *ATM,* une famille avec mutation dans *PMS2* et une famille avec mutation dans fumarate hydratase, chez des individus initialement négatifs pour *BRCA1–2.* Nous avons aussi constaté l'étonnante négativité de la recherche d'une mutation familiale *BRCA2* chez une jeune patiente atteinte d'un cancer du sein bilatéral, dont la mère était porteuse. Le séquençage de *BRCA1–2* s'est aussi révélé négatif, tout comme le test multigène par analyse de l'exome. Finalement, pour trois familles de souche canadienne-française, l'analyse d'un panel de 13 mutations fondatrices de *BRCA1–2* s'est avéré insuffisante puisque le séquençage a identifié d'autres mutations délétères.

Conclusion : De nombreux patients et leurs familles devraient bénéficier systématiquement d'un diagnostic ciblé, facilité par l'histoire familiale recherchant des conditions génétiques à risque de cancer du sein ou d'autres types de cancers (porteurs hétérozygotes de l'ataxie-télangiectasie et syndrome de Fanconi). Néanmoins, il demeure que pour un grand nombre de familles, les investigations utilisant une approche multigène par séquençage de nouvelle génération et analyse de l'exome devraient être considérées.