Australasian Colorectal Cancer Family Registry: a 15-year cohort

Mark Jenkins¹, Judi Maskell¹, Daniel Buchanan², Joanne Young³, Yoland Antill⁴, Julie Arnold⁵, Laura Baglietto⁶, Alex Boussioukas⁷,8, Mark Clendinning², James Dowty⁷, Michael Gattas⁷, Graham Giles⁷, Jack Goldblatt¹⁰, Louise Keogh¹¹, Judy Kirk¹², Barbara Leggett¹³,¹⁴,¹⁵, Lara Lipton¹⁶, Finlay Macrae¹⁷, Susan Parry¹⁸, Chris Rosty¹⁹, Melissa Southey⁰, John Stubbs²¹, Graeme Suthers²², Katherine Tucker²³, Michael Walsh², Aung Ko Win²⁴, Ingrid Winschipe²⁵, Graeme Young²⁶, Jeremy Jass²⁷, John Hopper²⁸

¹ Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville VIC; Cancer and Population Studies Group, Queensland Institute of Medical Research, Herston QLD; ² Familial Cancer Centre, Southern Health, Victoria; ³ New Zealand Family Environmental Cancer Study, Auckland City Hospital, Auckland, New Zealand; ⁴ Cancer Epidemiology Centre, Cancer Care Victoria, Clayton VIC; ⁵ Department of Medical and Cellular Pathology, The University of Melbourne, Parkville VIC; ⁶ Queen Elizabeth II Jubilee Hospital, Perth, WA; ⁷ Cancer Genomics and Proteomics, The University of Melbourne, Parkville VIC; ⁸ Queen Elizabeth II Health Sciences Centre, The Royal Adelaide Hospital, Adelaide, South Australia; ⁹ School of Medicine, The University of Queensland, Herston QLD; ¹⁰ Cancer Genomics and Proteomics, The University of Melbourne, Parkville VIC; ¹¹ Queen Elizabeth II Health Sciences Centre, The Royal Adelaide Hospital, Adelaide, South Australia; ¹² School of Medicine, The University of Queensland, Herston QLD; ¹³ Clinical Gastroenterology, Sir Charles Gairdner Hospital, Nedlands, WA; ¹⁴ Northern Sydney Area Health Service, Northern Sydney, NSW; ¹⁵ Clinical Gastroenterology, Sir Charles Gairdner Hospital, Nedlands, WA; ¹⁶ Victorian Clinical Pathology, The Royal Melbourne Hospital, Parkville VIC; ¹⁷ Department of Gastroenterology, Midcliff Hospital, Auckland, New Zealand; ¹⁸ Cancer Genomics and Proteomics, The University of Melbourne, Parkville VIC; ¹⁹ Department of Gastroenterology, Midcliff Hospital, Auckland, New Zealand; ²⁰ The Royal Melbourne Hospital, University of Melbourne, Parkville VIC; ²¹ Queen Elizabeth II Health Sciences Centre, The Royal Adelaide Hospital, Adelaide, South Australia; ²² Sydney, Australia; ²³ Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick NSW; ²⁴ Genetic Medicine, The Royal Melbourne Hospital, Parkville VIC; ²⁵ Freda Cancer Centre for Cancer Prevention and Control, Freda University, Adelaide SA; ²⁶ Department of Cellular Pathology, St Mark’s Hospital, Harrow, UK.

BACKGROUND

The Australasian Colorectal Cancer Family Registry (ACCFR) was established in 1997 and has been recruiting, surveying, collecting biospecimens, following-up, and genetically characterising for the past 15 years.

It constitutes one of the six registries of the NHMRC-funded International Colorectal Cancer Family Registry that was established as a resource for research into the genetic and environmental aetiology of colorectal cancer.

RECRUITMENT

Family Cancer Clinics

- Attendees to family cancer clinics with a family history of colorectal cancer, or being a member of a family known or suspected to be segregating a mutation in a mismatch repair or an MTHY gene.

Relatives: Probands and other participants were asked for permission to recruit first- and second-degree relatives of proband and additional family members of proband.

Community

- Self-nominated due to promotion or advertisement of ACCFR

Relatives: Probands and other participants were asked for permission to recruit first- and second-degree family members of proband.

Population-based

- Case probands: incident first primary colorectal cancer diagnosed in metropolitan Melbourne between 1996 and 2008 before age 50 years (100% attempted), or between age 51 and 60 (50% attempted).

Control probands: Age- and sex-matched to the cases from the electoral roll.

Probands: Relatives of proband were asked for permission to recruit first- and second-degree relatives of proband and any first-degree relatives of colorectal cancer affected participants (sequential ascertainment).

BASELINE PROTOCOL

- Interviews were administered in person or by telephone for all participants.
- Questionnaires were completed for lifestyle factors, family history of cancer and diet (see below).
- Blood samples (40 ml) were requested from selected participants.
- Tumour samples were sought from pathology laboratories and hospitals for all reported CRC and other Lynch syndrome cancers.

MOLECULAR CHARACTERISATION

Mismatch repair gene mutation testing

- Performed by Sanger sequencing or denaturing high performance liquid chromatography, followed by confirmation by capillary sequencing.
- Large duplication and deletion mutations were detected by Multiple Ligation Detection Probe Amplification.
- A pathogenic mutation was defined as a variant that was predicted to result in a stop codon, frameshift mutation, or large duplication or deletion, or a missense mutation previously reported within scientific literature and databases to be pathogenic.

PMH families

- Families with carriers of non-cancer
- Families with cancer

MMH families

- Families with carriers of non-cancer
- Families with cancer

MTHY gene mutation testing

- Genomic DNA extracted from each participant was sent to a central testing facility (Analytic Genetics Technology Centre, Toronto, Canada).

- DNA was screened for 9 variants of MTHY mutation: Y179C, G396D, Y104X, R727Q, E490K, C281X, 1114delC, c.333+2A>c., and c.1437_1439delAGA using the MassArray MALDI TOF Mass Spectrometry (MS) system (Sequenom, San Diego, CA).

- All samples with MS mobility shifts underwent screening of the entire MTHY coding region, promoter, and splice sites regions by denaturing high-performance liquid chromatography (Travanex Sequence 3500T STR System; Travanex, Omaha, NE; to confirm the mutation and to identify additional mutations.

- All MS-detected variants and WAVE mobility shifts were submitted for sequencing for mutation confirmation (ABI PRISM 3130, Applied Biosystems).

Tumour characterisation

Primary CRC tissue from the ACCFR: Jeremy Jeer Memorial Tissue Bank undertaken (methodology: GenePrint 10 loci) Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville VIC.

- CRCs from the probands were characterised for MMR-deficiency by: 1. microsatellite instability testing (MSI) using a ten-marker panel with tumours with 30% or more of the markers shown to be unstable were declared to have high levels of microsatellite instability (MSI-H).

- 2. by immunohistochemistry (IHC) for the expression of the four MMR proteins.

- CRCs demonstrating loss of expression of the MLH1 and PMS2 proteins by IHC were referred for MLH1 gene promoter testing using MethyLight assay.

- All proband CRCs were tested for the BRAF p.V600E somatic mutation using allele specific PCR.

- All CRCs tested by IHC for the expression of the four MMR proteins.

- Patients tested for MSI and MTHY mutations.

Follow-up

- Active Follow-up
- Follow-up of all families (except population-based control families) attempted every 5 years.
- A mixture of methods was used. Participants were asked to complete a telephone interview or were mailed a questionnaire to complete with phone follow-up for additional information and for family history of cancer.

- The mailed questionnaire was asked to ask major details on cancer diagnoses, screening, surgery, family history and genetic testing.

- Medical records were sought to verify all reported CRC and other Lynch syndrome cancers. On average, 110 reported cancer were verified per year.

- Medical records were sought to verify all reported polyps. On average, 108 reported polyps were verified per year.

- Blood samples were requested for participants with new diagnoses of CRC and other Lynch syndrome cancers, and new recurrences in existing families, and for whom DNA samples needed sampling. In total, 210 blood samples were collected each year of follow-up.

- Passive Follow-up
- All family members (participants and non-participants) were linked to the National Cancer Clearing House to verify and update cancer diagnoses and vital status.

- All family members (participants and non-participants) who lived in Victoria were linked to the Victorian Cancer Registry to verify and update cancer diagnoses and vital status.

- All family members (participants and non-participants) currently being linked to the National Cancer Clearing House to verify and update cancer diagnoses.

PROGRESS

- Follow-up status 1st follow-up 2nd Follow-up

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<th>Deceased</th>
<th>Lost</th>
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Note: This does not include population-based control families.

FOLLOW-UP

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<th>Other New Ca</th>
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<th>Deaths</th>
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</table>

TOTAL | 10467 | 2811 | 426 | 2623 | 755 | |

Note: This does not include population-based control families.

FEEDBACK OF TEST RESULTS

- Participants of MMR families detected by ACCFR are given the opportunity to learn of their genetic status via a clinical genetics service.

- The ACCFR has accordingly written to 1622 participants of 330 families segregating a mutation in a mismatch repair gene or an MTHY gene.

- The ACCFR follow the progress of participants through the counselling process to determine how many attend a clinic and learn of their results.

CONCLUSIONS

- The ACCFR is the largest and best characterised colorectal cancer family registry in the world and has been a major contributor to colorectal cancer research through the Colon Cancer Family Registry.

- We have demonstrated that, using a prospective family-study cohort design, we can achieve very high response over a decade of follow-up.

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