Australasian Colorectal Cancer Family Registry: a 15-year cohort

Mark Jenkins[1], Judi Maskiell[1], Daniel Buchanan[2], Joanne Young[2],Yoland Antill[3], Julie Arnold[4], Laura Baglietto[5], Alex Boussioutas[6,7,8], Mark Clendenning[2], James Dowty[1], Michael Gattas[9], Graham Giles[5], Jack Goldblatt[10], Louise Keogh[11], Judy Kirk[12], Barbara Leggett[13,14,15], Lara Lipton[16], Finlay Macrae[17], Susan Parry[4,18], Christophe Rosty[2,19], Melissa Southey[20], John Stubbs[21], Graeme Suthers[22,23], Katherine Tucker[24], Michael Walsh[2], Aung Ko Win[1], Ingrid Winship[6,25], Graeme Young[26], Jeremy Jass[27], John Hopper[1].

[1] Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville VIC; [2] Cancer and Population Studies Group, Queensland Institute of Medical Research, Herston QLD; [3] Familial Cancer Centre, Southern Health VIC; [4] New Zealand Familial Gastrointestinal Cancer Registry, Auckland City Hospital, Auckland, New Zealand; [5] Cancer Epidemiology Centre, Cancer Council Victoria, Carlton VIC; [6] Department of Medicine, Royal Melbourne Hospital, The University of Melbourne, Parkville VIC; [7] Cancer Genomics and Predictive Medicine, Peter MacCallum Cancer Centre, East Melbourne VIC; [8] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville VIC; [9] Queensland Clinical Genetics Service, Royal Childrens' Hospital, Herston QLD; [10] Genetic Services of Western Australia and School of Paediatrics and Child Health, University of Western Australia, Perth, WA; [11] Centre for Women's Health, Gender & Society, University of Melbourne, Melbourne VIC; [12] Westmead Hospital, The University of Sydney, NSW; [13] Department of Gastroenterology and Hepatology, The Royal Brisbane and Women's Hospital, Brisbane QLD; [14] Conjoint Gastroenterology Laboratory, Pathology Queensland, Clinical Research Centre of Royal Brisbane & Women´s Hospital Research Foundation, Queensland Institute of Medical Research, Herston QLD; [15] School of Medicine, University of Queensland, Herston QLD; [16] Ludwig Institute for Cancer Research, The Royal Melbourne Hospital, Parkville VIC; [17] Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Parkville VIC; [18] Department of Gastroenterology, Middlemore Hospital, Auckland, New Zealand; [19] Department of Molecular and Cellular Pathology, University of Queensland, Herston QLD; [20] Genetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Parkville VIC; [21] Cancer Voices Australia, Sydney NSW; [22] Department of Paediatrics, University of Adelaide, SA; [23] South Australian Clinical Genetics Service, North Adelaide SA; [24] Hereditary Cancer Clinic, Prince of Wales Hospital, Randwick NSW; [25] Genetic Medicine, The Royal Melbourne Hospital, Parkville VIC; [26] Flinders Centre for Cancer Prevention and Control, Flinders University, Adelaide SA; [27] Department of Cellular Pathology, St Marks 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 NIH funded international Colon Cancer Family Registry that was established as a resource for research into the genetic and environmental aetiology of colorectal cancer.

RECRUITMENT

Family Cancer Clinics

Probands: 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 *MUTYH* gene.

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

Community

Probands: Self- nominated due to promotion or advertisement of ACCFR

Relatives: Probands and other participants were asked for permission to recruit first- second- and additional 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 cases from the electoral roll

Relatives: Probands and other participants were asked for permission to recruit first- 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 mls) were requested from selected participants.
- Tumour samples were sought from pathology laboratories and hospitals for all reported CRC and other Lynch syndrome cancers.

Risk factor	Items
Demographics	Age, date of birth, sex, twin status, marriage status, education, self- and ancestor's country of birth, yers in Australia, suburb, self and ancestor's ethnicity, self and ancestor's religion,
Bowel cancer tests	**FOBT, sigmoidoscopy, colonoscopy, (number, age at first and last, reasons for first test),**
Polyps	**Age at first and last and type of polyp, age at first and last polypectomy**
Bowel conditions	FAP, Crohn's disease, ulcerative colitis, irritable bowel syndrome, diverticular disease (ages at diagnosis)
Bowel surgery	**Amount removed (number of and ages at of surgeries), gall bladder removed (age at surgery)**
Diabetes	Age at diagnosis, medication use (type of medication and years of use)
High blood fats	Cholesterol, triglycerides (age at diagnosis, years and ages of medication use)
Cancer history	**Sites, ages at diagnosis, radiation therapy**
Medication use	Aspirin, paracetamol, anti-inflammatories, laxatives, antacids, calcium supplements, multivitamins, folate (frequency timing and number of years of regular use)
Reproductive history	Age at menarche, number of pregnancies and births, age at first and last birth.
Hormone use	Oral contraceptives, hormone replacement therapy (age at first and last use, number of years, oestrogen and/or progesterone, tamoxifen and/or raloxifene)
Food	Method and frequency of meat cooking, 121 item food frequency and portion size questionnaire.
Physical activity	Specified and open ended types by exersion level (hours per week, number of weeks per year, number of years) for 20s, 30s-40s, and since 50s.
Alcohol consumption	Beer (light, heavy), wine, cider, sprits (number of drinks, frequency, total months/years drinking) for 20s, 30-40s, and since 50s.
Smoking	Cigarettes, cigars (age began, age last used, total number of years, cigarettes/cigars per day)
Height /weight	Weight (current and 18-20 years old), current height
Genetic testing	**Attended research of familial cancer or attended a cancer family clinic.**
First- and second-degree relatives	**Age, sex, date of birth, date of death, cancer diagnoses (type of cancer and ages of diagnoses).**

Note: Bold items used in follow-up.

RESOURCE

	Families	Individuals		
		Cancer and demographic	Risk factor Questionnaires	Blood Samples
Family Cancer Clinics				
Adelaide	127	5338	1265	1170
Auckland	72	2689	586	523
Brisbane	95	4881	1203	1172
Perth	113	4552	796	696
Melbourne	94	4678	800	645
Sydney	76	4024	754	607
Community	5	250	34	27
Population-based				
Cases	959	45911	5021	3092
Controls	271	6466	1053	272
TOTAL	**1812**	**78789**	**11512**	**8204**

Current cancer status of participant	Blood or saliva sample	Tumour sample
Colorectal cancer	1885	1,668
Other cancer	919	265
No cancer	5400	0
Total	**8,204**	**1,933**

MOLECULAR CHARACTERISATION

Mismatch repair gene mutation testing

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

MMR	Families	Confirmed carriers	Confirmed non-carriers	Carriers with cancer at enrolment	Carriers without cancer at enrolment	Carriers with baseline risk factor questionnaire
MLH1	110	350	427	98	252	253
MSH2	132	453	544	123	330	340
MSH6	40	94	90	41	53	86
PMS2	25	57	68	24	33	48
Total	**307**	**954**	**1129**	**286**	**668**	**727**

***MUTYH* 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 *MUTYH* mutations: Y179C, G396D, Y104X, R274Q, E480X, Q391X, c.1147delC, c.933+3A>C, and c.1437_1439delGGA, using the MassArray MALDI-TOF Mass Spectrometry (MS) system (Sequenom, San Diego, CA).
- All samples with MS mobility shifts underwent screening of the entire *MUTYH* coding region, promoter, and splice sites regions by denaturing high-performance liquid chromatography (Transgenomic Wave 3500HT System; Transgenomic, 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 3130XL Genetic Analyser).

MUTYH	Confirmed carriers	Carriers with cancer at enrolment	Carriers without cancer at enrolment	Carriers with baseline risk factor questionnaire
monoallelic	76	26	50	72
biallelic	12	7	5	9
Total	**88**	**33**	**55**	**81**

Tumour characterisation

Primary CRC tissue from the ACCFR Jeremy Jass Memorial Tissue Bank underwent pathology review and molecular characterisation as follows.

- CRCs from the probands were characterised for MMR-deficiency by:
  1. microsatellite instability testing (MSI) using a ten-marker panel where tumours with 30% or more of the markers show instability were considered to have high levels of microsatellite instability (MSI-H) and
  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 subsequently characterised for methylation of the MLH1 gene promoter using a MethyLight assay.
- All proband CRCs were tested for the BRAF p.V600E somatic mutation using allele-specific PCR.
- All CRCs were reviewed by specialist GI pathologists for tumour site, grade, margin, presence of mucinous component, peritumoral lymphocytes, Crohn's-like lymphocytic reaction, tumour-infiltrating lymphocytes, and synchronous CRC.

Test	Participants
Pathology reviews completed	1113
Tumors tested for MSI	798
Tumors tested for MMR IHC	1113
Tumors tested for MLH1 methylation	133
Tumors tested for *BRAF* V600E mutation	1074

FOLLOW-UP

Active Follow-up

- Follow-up of all families (except population-based control families) attempted approximately 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 abbreviated 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 cancer. 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 recruits in existing families, and for whom DNA samples needed replenishing. On average, 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 Death Index to verify vital status and causes of death.
- 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
Completed	8211	4256
Refused	528	230
Deceased	584	174
Lost	130	48
Pending	*1014*	*4517*
Participation	**93%**	**91%**

Note: This does not include population-based control families

Cancer Status at enrollment	Completed baseline	Completed "5-year" follow-up	Completed "10-year" follow-up	Person-years of follow-up
Colorectal cancer	1802	1296	654	9750
Other cancer	665	487	241	3640
No cancer	8000	6428	3361	48945
TOTAL	**10467**	**8211**	**4256**	**62335**

Note: This does not include population-based control families

Cancer Status at enrollment	New CRC N (incidence)	New Other Ca N (incidence)	New Polyps N (incidence)	Deaths N (incidence)
Colorectal cancer	97 13/1000 py	141 19/1000 py	522 69/1000 py	382 51/1000 py
Other cancer	20 8/1000 py	60 24/1000 py	132 52/1000 py	93 37/1000 py
No cancer	86 3/1000 py	211 6/1000 py	1211 36/1000 py	280 8/1000 py
TOTAL	**203**	**412**	**1865**	**755**

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 *MUTYH* 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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