Characterisation of Familial Colorectal Cancer Type X, Lynch syndrome, and non-familial colorectal cancer

S Shiovitz1,2, W K Copeland3, M N Passarelli3, A N Burnett-Hartman3,4, W M Grady1,2,3, J D Potter3,4,5, S Gallinger6, D D Buchanan7,8, C Rosty8,9,10, A K Win7, M Jenkins7, S N Thibodeau11, R Haile12, J A Baron13, L L Marchand14, P A Newcomb3,4 and N M Lindor15 for the Colon Cancer Family Registry

1Department of Medicine, University of Washington, Seattle, WA, USA; 2Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 3Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 4Department of Epidemiology, University of Washington, Seattle, WA, USA; 5Centre for Public Health Research, Massey University, Wellington, New Zealand; 6Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 7University of Melbourne, Parkville, VIC, Australia; 8Cancer and Population Studies Group, Queensland Institute of Medical Research, Brisbane, QLD, Australia; 9University of Queensland, School of Medicine, Herston, QLD, Australia; 10Envoi Pathology, Herston, QLD, Australia; 11Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; 12Stanford Cancer Institute, Palo Alto, CA, USA; 13Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC, USA; 14University of Hawaii Cancer Center, Honolulu, HI, USA and 15Department of Health Science Research, Mayo Clinic, Scottsdale, AZ, USA

Background: Familial Colorectal Cancer Type X (FCCTX) is defined as individuals with colorectal cancer (CRC) who families meet Amsterdam Criteria-1 (AC1), but whose tumours are DNA-mismatch-repair-proficient, unlike Lynch syndrome (LS). FCCTX does not have an increased risk of extra-colonic cancers. This analysis compares epidemiologic and clinicopathologic features among FCCTX, LS, and ‘non-familial’ (non-AC1) CRC cases.

Methods: From the Colon Cancer Family Registry, FCCTX (n = 173), LS (n = 303), and non-AC1 (n = 9603) CRC cases were identified. Questionnaire-based epidemiologic information and CRC pathologic features were compared across case groups using polytomous logistic regression.

Results: Compared with LS, FCCTX cases were less likely to be current (vs never) smokers; have a proximal subsite (vs rectal) tumour; or have mucinous histology, poor differentiation, or tumour-infiltrating lymphocytes. There were no observed differences in co-morbidities or medication usage.

Conclusions: FCCTX were less likely to be current tobacco users; other exposures were similar between these groups. Histopathologic differences highly suggestive of LS CRCs do not appear to be shared by FCCTX.

‘Familial Colorectal Cancer Type X’ (FCCTX) collectively describes cases of colorectal cancer (CRC) that meet clinical Amsterdam Criteria-1 (AC1) for Lynch syndrome (LS), but whose tumours are DNA-mismatch-repair-proficient as assessed by tumour immuno-histochemistry (IHC) and/or microsatellite instability (MSI) testing (Vasen et al, 1991; Lindor et al, 2005). Approximately half of CRC cases who meet AC1 (three relatives with CRC across two successive generations (with one case being a first-degree relative of the other two), at least one case diagnosed before age 50, and the exclusion of familial adenomatous polyposis), are now classified as...
Characterising Familial Colorectal Cancer Type X

### MATERIALS AND METHODS

As described elsewhere (Newcomb et al. 2007), the CCFR (http://coloncfr.org) is an international consortium of CRC cases and controls from population- and/or clinic-based sites in North America and Australasia. Recruited during 1998–2007, participants completed written informed consent for study enrolment; protocols were approved by local institutional review boards. Collection of epidemiologic and family history data and biospecimens was standardised across all centres.

The following tumour characteristics were abstracted from the clinical histopathology report and/or from pathologist review: location, size, nodal status, differentiation, histologic type, and presence/absence of peritumoural lymphocytes, Crohn’s-like reaction, tumour-infiltrating lymphocytes, and venous invasion. MSI and/or IHC were performed on all tumour samples (Lindor et al., 2005; Newcomb et al., 2007).

Cases were allocated to one of the three groups: (1) ‘LS’ (n = 312) for cases meeting AC1 and whose tumours were classified as MSI (MSI-high and/or MMR-deficient), (2) ‘FCCTX’ (n = 177) for cases meeting AC1, but with non-MSI tumours, or (3) ‘non-AC1’ (n = 12,175) for the remainder of CRC cases whose family histories did not meet AC1. No more than one individual per family was included in the analysis. Restricting the analysis to available epidemiologic/tumour information, we included 173/146 FCCTX, 303/245 LS, and 9,603/7,878 non-AC1 CRC cases.

### Statistical methods.

Odds ratios (OR) with 95% confidence intervals were estimated using polytomous logistic regression models. All models are adjusted for age at diagnosis, sex, and study site.

### Table 1. Epidemiologic characteristics of FCCTX compared with Lynch syndrome and non-Amsterdam Criteria-1 colorectal cases in the Colon Cancer Family Registry

| Characteristic, n (%) | FCCTX (n = 173) | Lynch (n = 303) | Non-AC1 (n = 9,603) | FCCTX vs Lynch* ORb (95% CI) | FCCTX vs non-AC1b ORb (95% CI) | Lynch vs non-AC1b ORb (95% CI) |
|----------------------|----------------|----------------|---------------------|-----------------------------|-----------------------------|-----------------------------|
| **Case characteristics**<sup>a</sup> | | | | | | |
| Age, mean (s.d.) | 53.3 (11.3) | 50.5 (11.4) | 56.3 (12.0) | 1.02 (1.00, 1.03) | 0.99 (0.98, 1.00) | 0.97 (0.96, 0.98) |
| Male gender | 76 (44%) | 149 (49%) | 4797 (50%) | 0.81 (0.56, 1.18) | 0.80 (0.59, 1.09) | 0.99 (0.79, 1.25) |
| BMI, mean (s.d.) | 27.5 (6.6) | 26.5 (5.8) | 27.2 (5.8) | 1.03 (1.00, 1.07) | 1.02 (0.99, 1.04) | 0.98 (0.96, 1.01) |
| **Smoking** | | | | | | |
| Never | 85 (49%) | 148 (49%) | 4252 (45%) | 1.17 (0.78, 1.76) | 0.99 (0.72, 1.37) | 0.85 (0.65, 1.11) |
| Former | 74 (43%) | 106 (35%) | 4198 (44%) | 1.0 (Reference) | 1.0 (Reference) | 1.0 (Reference) |
| Current | 13 (8%) | 49 (16%) | 1096 (11%) | 0.48 (0.24, 0.94) | 0.62 (0.35, 1.13) | 1.30 (0.93, 1.83) |
| **Co-morbidities (yes/no) (% yes)** | | | | | | |
| Diabetes | 17/155 (9%) | 79/222 (36%) | 1154/8404 (12%) | 1.04 (1.00, 1.08) | 1.16 (1.10, 1.23) | 0.78 (0.74, 0.82) |
| Hyperlipidemia | 42/129 (24%) | 2995/6530 (31%) | 0.84 (0.53, 1.31) | 0.88 (0.61, 1.27) | 1.05 (0.80, 1.39) | 1.19 (0.89, 1.60) |
| Asthma | 38/134 (22%) | 2771/6747 (29%) | 0.83 (0.52, 1.34) | 0.99 (0.68, 1.46) | 1.13 (0.83, 1.55) | 0.78 (0.65, 0.93) |
| Acetaminophen | 23/149 (13%) | 1469/8026 (15%) | 0.72 (0.42, 1.23) | 0.81 (0.52, 1.27) | 1.14 (0.84, 1.56) | 1.14 (0.83, 1.55) |
| NSAIDs | 31/141 (22%) | 1555/7910 (16%) | 1.01 (0.62, 1.66) | 1.16 (0.78, 1.73) | 1.10 (0.75, 1.61) | 1.10 (0.75, 1.61) |
| Laxatives | 38/134 (22%) | 271/6747 (29%) | 1.04 (0.65, 1.65) | 1.04 (0.72, 1.51) | 1.01 (0.75, 1.36) | 1.01 (0.75, 1.36) |
| Multivitamin | 39/133 (23%) | 2136/7375 (22%) | 0.88 (0.60, 1.31) | 0.98 (0.71, 1.43) | 1.06 (0.78, 1.45) | 1.06 (0.78, 1.45) |
| Folic acid | 72/140 (32%) | 4829/7402 (50%) | 1.03 (0.58, 1.84) | 1.13 (0.70, 1.80) | 1.09 (0.76, 1.57) | 1.09 (0.76, 1.57) |
| Calcium | 36/136 (27%) | 2507/7012 (26%) | 0.84 (0.51, 1.38) | 0.80 (0.54, 1.19) | 0.95 (0.70, 1.30) | 0.95 (0.70, 1.30) |
| **Female (yes/no) (% yes)** | | | | | | |
| Oral hormonal contraceptives | 70/72 (77%) | 115/39 (35%) | 2783/1972 (58%) | 0.93 (0.50, 1.72) | 1.31 (0.81, 2.12) | 1.41 (0.95, 2.09) |
| PMH with uterus intact | 23/41 (56%) | 1041/1805 (36%) | 0.44 (0.05, 3.92) | 0.90 (0.12, 6.80) | 2.04 (0.76, 5.42) | 2.04 (0.76, 5.42) |
| PMH with hysterectomy | 6/12 (33%) | 314/471 (39%) | 0.68 (0.35, 1.34) | 1.19 (0.68, 2.09) | 1.76 (1.17, 2.64) | 1.76 (1.17, 2.64) |

<sup>a</sup> Abbreviations: AC1 – Amsterdam Criteria-1; BMI – body mass index; FCCTX – Familial Colorectal Cancer Type X; PMH – post-menopausal hormone use; s.d. – standard deviation.

<sup>b</sup> OR per one unit increase in continuous variables (age at diagnosis and BMI). For binary variables, the reference group is those without the characteristic.

<sup>c</sup> Age at diagnosis (years); BMI = body mass index; FCCTX = Familial Colorectal Cancer Type X; PMH = post-menopausal hormone use; s.d. = standard deviation.

<sup>d</sup> All models are adjusted for age at diagnosis, sex, and study site.
Table 2. Histopathologic characteristics of FCCTX in CCFR compared with Lynch syndrome and non-Amsterdam Criteria-1 colorectal cases

| Characteristic, n (%) | FCCTX (n = 146) | Lynch (n = 245) | Non-AC1 (n = 7,878) | FCCTX vs Lynch* OR (95% CI) | FCCTX vs non-AC1* OR (95% CI) | Lynch vs non-AC1* OR (95% CI) |
|----------------------|-----------------|--------------|-------------------|---------------------------|---------------------------|---------------------------|
| **Cancer subsite**   |                 |              |                   |                           |                           |                           |
| Caecum               | 17 (12%)        | 55 (22%)     | 1034 (13%)        | 0.18 (0.09, 0.37)         | 0.95 (0.54, 1.67)         | 5.2 (3.32, 8.14)          |
| Ascending            | 15 (10%)        | 64 (26%)     | 1129 (14%)        | 0.13 (0.06, 0.27)         | 0.66 (0.35, 1.22)         | 5.22 (3.37, 8.07)         |
| Transverse           | 13 (9%)         | 31 (13%)     | 578 (7%)          | 0.27 (0.12, 0.59)         | 1.18 (0.63, 2.21)         | 4.42 (2.67, 7.41)         |
| Descending           | 7 (5%)          | 14 (6%)      | 438 (6%)          | 0.38 (0.14, 1.08)         | 0.78 (0.35, 1.74)         | 2.03 (1.03, 3.99)         |
| Sigmod               | 37 (25%)        | 22 (9%)      | 1931 (25%)        | 1.02 (0.51, 2.04)         | 0.98 (0.63, 1.52)         | 0.96 (0.55, 1.66)         |
| Rectum               | 50 (34%)        | 37 (15%)     | 2455 (31%)        | 1 (Reference)             | 1 (Reference)             | 1 (Reference)             |
| Missing/Subsite      | 7 (5%)          | 22 (9%)      | 313 (4%)          |                           |                           |                           |
| T-stage              |                 |              |                   |                           |                           |                           |
| T1                   | 22 (15%)        | 27 (11%)     | 958 (12%)         | 1 (Reference)             | 1 (Reference)             | 1 (Reference)             |
| T2                   | 26 (18%)        | 48 (20%)     | 1328 (17%)        | 0.75 (0.35, 1.57)         | 0.78 (0.43, 1.40)         | 1.04 (0.64, 1.70)         |
| T3                   | 74 (51%)        | 127 (52%)    | 4242 (54%)        | 0.85 (0.45, 1.63)         | 0.65 (0.39, 1.07)         | 0.76 (0.49, 1.18)         |
| T4                   | 11 (8%)         | 9 (4%)       | 576 (7%)          | 1.75 (0.60, 5.05)         | 0.72 (0.34, 1.52)         | 0.41 (0.19, 0.89)         |
| Missing/Subsite      | 13 (8%)         | 34 (13%)     | 774 (10%)         |                           |                           |                           |
| **Histology**        |                 |              |                   |                           |                           |                           |
| Adenocarcinoma       | 132 (90%)       | 191 (78%)    | 6771 (86%)        | 1 (Reference)             | 1 (Reference)             | 1 (Reference)             |
| Mucinous             | 11 (8%)         | 41 (17%)     | 856 (11%)         | 0.39 (0.19, 0.80)         | 0.62 (0.38, 1.02)         | 0.70 (0.43, 1.15)         |
| Signet ring          | 4 (3%)          | 2 (1%)       | 3 (1%)            | 0.38 (0.04, 3.44)         | 0.41 (0.21, 0.82)         | 1.25 (0.73, 2.14)         |
| Missing/Subsite      | 2 (1%)          | 4 (2%)       | 172 (2%)          |                           |                           |                           |
| **Additional features (yes/no) (% yes)**b
| Peritumoural lymphocytes | 23/57 (28%) | 61/71 (46%) | 954/1608 (37%) | 0.49 (0.26, 0.90)         | 0.75 (0.45, 1.25)         | 1.54 (1.07, 2.23)         |
| Crohn’s-like lymphocytes | 14/60 (19%) | 61/70 (37%) | 627/1843 (25%) | 0.27 (0.14, 0.54)         | 0.78 (0.43, 1.41)         | 2.84 (1.97, 4.10)         |
| Tumour-infiltrating lymphocytes | 20/65 (24%) | 98/43 (70%) | 704/1920 (27%) | 0.14 (0.07, 0.26)         | 0.89 (0.53, 1.50)         | 6.41 (4.40, 9.36)         |
| Venous invasion      | 16/79 (11%)    | 9/135 (4%)   | 735/4027 (9%)     | 3.21 (1.35, 7.65)         | 1.27 (0.73, 2.21)         | 0.40 (0.20, 0.79)         |

** Abbreviations: AC1 = Amsterdam Criteria-1; FCCTX = Familial Colorectal Cancer Type X.

*a All models are adjusted for age at diagnosis, sex, and study site. Reference group is those without the characteristic.

b These features were only collected at four of six study sites (Mayo Clinic, Australasia, UH, and CCO).

RESULTS

FCCTX cases were slightly older at diagnosis than LS (mean 53.3 vs 50.5 years Table 1). By definition, all FCCTX and LS families met AC-1. In comparison, of the 9603 individuals who were classified as non-AC1, 33% were diagnosed before age 50, 5% had two or more first-degree relatives with CRC (13% had one first-degree relative), and 15% had MSI-high tumours.

The self-reported proportion of ever smokers was similar across the three groups, but FCCTX had the lowest prevalence of current smokers (P = 0.03 and 0.12 compared with LS and non-AC1, respectively). A higher proportion of FCCTX vs LS reported being former smokers. BMI and the prevalence of diabetes, hyperlipidemia, aspirin/NSAID and other medication usage, and gynaecologic history elements did not vary significantly between groups.

FCCTX CRCs were less often located in proximal subsites than LS (caecal, ascending, or transverse colon; all P < 0.001, Table 2); no subsite difference was observed for FCCTX vs non-AC1 tumours. Overall, the LS group had the lowest proportion of T4 tumours, but a statistically significant difference was observed only when comparing LS and non-AC1 CRCs. Nodal N-stage could not be reliably assessed owing to the variability in missing data between sites (data not shown).

FCCTX CRCs were more commonly poorly differentiated compared with LS/non-AC1 tumours, and were less often mucinous than LS tumours. FCCTX tumours had a smaller proportion of peritumoural lymphocytes, Crohn’s-like reaction,

DOI: 10.1038/bjc.2014.309
and TIL than LS CRCs, but there was no difference compared with non-AC1 tumours. Venous invasion was most commonly seen in FCCTX tumours.

**DISCUSSION**

This study evaluated epidemiologic and clinicopathologic data across FCCTX, LS, and non-AC1 cases of CRC. A statistically significant difference across these groups was noted for smoking history, whereas no differences were observed in co-morbidities, medication use, or gynaecologic history elements. Classic histopathologic features of LS CRCs were much less commonly observed in FCCTX CRCs. There were no clear distinguishing features for FCCTX vs non-AC1 tumours.

**Comparison of FCCTX and LS.** Individuals classified as FCCTX were less likely to be current smokers. Tobacco use is associated with a higher incidence of colorectal adenoma and invasive CRC in both the general population and in LS (Watson *et al.*, 2004; Botteri *et al.*, 2008; Pande *et al.*, 2010). The difference in CRC prevalence between FCCTX and LS may be partially mediated by differences in tobacco use habits, although we cannot rule out that smoking has less effect in FCCTX than in LS.

In this comprehensive pathologic analysis, we confirmed the previously-reported left- vs right-sided predominance of FCCTX vs LS by subsite (Llor *et al.*, 2005; Mueller-Koch *et al.*, 2005; Valle *et al.*, 2007). In the present analysis, there were also a greater proportion of large (T4) primary FCCTX tumours compared with LS CRCs. On histologic review, the mucinous histology, poor differentiation, and TIL features reported as characteristic of LS tumours (Jenkins *et al.*, 2004; Valle *et al.*, 2007; Chen *et al.*, 2008; Klarskov *et al.*, 2011; Koh *et al.*, 2011; Klarskov *et al.*, 2012) FCCTX CRCs in our analysis also had a lower proportion for peritumoural lymphocytes and Crohn’s-like reaction, but a significantly higher proportion had venous invasion relative to that observed in LS CRCs.

**Comparison of FCCTX and non-AC1.** Epidemiologic factors did not distinguish FCCTX and non-AC1 cases. FCCTX tumours had significantly lower frequency of poor differentiation than non-AC1 CRCs and a trend toward a higher proportion with venous invasion. Tumour subsite, T-stage, and tumoural lymphocytes were not observed to differ between FCCTX and non-AC1 tumours.

**Strengths and limitations.** This analysis benefits from a large, international cohort of patients with standardised data collection, providing the opportunity to compare FCCTX, LS, and non-AC1 CRCs in the first epidemiologic and in-depth clinicopathologic analysis. The epidemiologic features were assessed by a single baseline survey, as it is difficult to longitudinally evaluate any changes in these factors in such rare syndromes. To keep this analysis meaningful, we selected a classification based on the current information readily available to clinicians, namely personal and family history (the Amsterdam Criteria) and standard tumour analysis (MSI and/or MMR IHC). The non-AC1 cohort contains a mixture of sporadic MSI (typically due to MLH1 promoter hypermethylation and associated sporadic BRAF mutations (Lynch *et al.*, 2007)) and non-MSI cases. CRC is increasingly being recognised as genetically and epigenetically heterogeneous (Marisa *et al.*, 2013), making selection of a true comparison group difficult. FCCTX is likely also genetically heterogeneous and would benefit from in-depth molecular characterisation (Abdel-Rahman *et al.*, 2005; Sanchez-de-Abajo *et al.*, 2007; Goel *et al.*, 2010). It should also be noted that it is possible, given the multiple statistical comparisons performed in this analysis, that the noted associations could be chance findings. Thus, independent validation is needed.

**CONCLUSIONS**

This study compared FCCTX, LS, and non-AC1 CRC cases. FCCTX were less likely to be current tobacco users; other exposures were similar between these groups. Subsite analysis confirms the distal colonic predominance of FCCTX vs LS CRCs. Histopathologically, mucinous histology, poor differentiation, and TIL were strongly associated with LS, rather than FCCTX or non-AC1, tumours, whereas venous invasion was more commonly seen in FCCTX. Additional molecular analysis may eventually explain the observed histopathologic differences between FCCTX and LS tumours.

**REFERENCES**

Abdel-Rahman WM, Ollikainen M, Kariola R, Jarvinen HJ, Mecklin JP, Nystrom-Lahti M, Knuutila S, Peltonaki P (2005) Comprehensive characterisation of HNPCC-related colorectal cancers reveals striking molecular features in families with no germline mismatch repair gene mutations. *Oncogene* 24(9): 1542–1551.

Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P (2008) Smoking and colorectal cancer: a meta-analysis. *JAMA* 300(23): 2765–2778.

Chen JR, Changhien CR, Chen JS, Tang RP, Wang Y (2008) Mismatch repair protein expression in Amsterdam II criteria-positive patients in Taiwan. *Br J Surg* 95(1): 102–110.

Dove-Edwin I, de Jong AE, Adams J, Mesher D, Lipton L, Sasieni P, Vasaen HF, Thomas HJ (2006) Prospective results of surveillance colonoscopy in dominant familial colorectal cancer with and without Lynch syndrome. *Gastroenterology* 130(7): 1995–2000.
Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)