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[1]

Amsterdam-Defined Asian HNPCC Patients: Data From the Singapore Polyposis Registry

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Key words: Asians, HNPCC, Amsterdam criteria

Background: Routine genetic testing is costly and not widely available in Asia. A thorough family history remains the most important and cost-effective means of diagnosing hereditary non-polyposis colorectal cancer (HNPCC). However, the usefulness of the Amsterdam criteria for diagnosis of HNPCC in Asians has not been thoroughly evaluated. This study aims to characterize the phenotype of Amsterdam-defined Asian HNPCC patients registered with the Singapore Polyposis Registry since 1989.

Methods: A review was conducted of HNPCC patients registered with the Singapore Polyposis Registry over a 16-year period. All patients fulfilled the Amsterdam I and II criteria. Data on demographics, site of colorectal cancers, synchronous cancers, associated extra-colonic cancers in pedigrees, histology, type of colonic resections, Dukes' and TNM staging were obtained from a prospective computerized database.

Results: A total of 52 patients with colorectal cancer from Amsterdam-defined HNPCC families were reviewed. The male to female ratio was 1.6:1 with median age of 44.5 years (range 27-73) at diagnosis of first cancer. The ethnic distribution was 47 Chinese (91%) and 5 Malays (9%), and the median follow up was 44.9 months (range 2-183 months). More than two thirds (69%) of the tumours were left-sided, with the majority located in the sigmoid colon. More than half (60%) of the tumours presented at a late stage (Dukes' C&D), with 83% being moderately or poorly differentiated adenocarcinomas. Left-sided tumours tend to have more advanced Duke's stage disease ($p=0.096$) and a higher rate of metastasis ($p=0.08$) compared to right-sided lesions. There was, however, no significant difference in disease-free or overall survival between left and right-sided tumours.

Conclusions: In contrast to data from studies on Caucasian populations, Amsterdam-defined Asian HNPCC families appeared to have more left-sided tumours. These differences may be due to inadequacies of the Amsterdam criteria when applied to an Asian population or a true ethnic variation in HNPCC phenotypic expression. Further studies are needed to clarify the genotypic-phenotypic correlations and the usefulness of the Amsterdam criteria in diagnosis of HNPCC in Asian populations.

[2]

Endoscopic Surveillance and Surgical Intervention Can Reduce Duodenal Malignancy in Familial Adenomatous Polyposis (FAP) Patients

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Key words: familial adenomatous polyposis, duodenal adenomas, pancreas-sparing duodenectomy

Background: Endoscopic surveillance and Spigelman classification is used to determine the need for surgical resection.

Methods: FAP patients undergoing surveillance and surgical intervention over the past 15 years were reviewed.

Results: One-hundred sixty-three patients underwent upper endoscopic surveillance. Eighty-four patients were male and the mean age was 48.3 ± 14.4 years. Six-hundred ninety-six endoscopies were performed, averaging 5.7 per patient with a mean follow-up of 100.6 months. Spigelman stage III/IV was present in 53 (33%) patients at initial endoscopy. During endoscopic surveillance 11/64 (17%) stage I and 18/39 (46%) stage II patients progressed to Spigelman III/IV, over a mean of 10.5 and 7.6 years, respectively. Thirty patients (18%) underwent endoscopic therapy: 11 endoscopic polypectomies (EP), 14 argon/electro-coagulation (APC) and

5 combined. Ten patients went on to surgical intervention. One patient was downstaged from Spigelman IV to II after 17 combined endoscopic procedures. There were 5 endoscopic related complications (0.7%) in the surveillance group: 2 duodenal perforations, one ileus, one pancreatitis, and one post-procedural abdominal pain.

Forty-seven operations were performed for Spigelman III/IV polyposis: 30 pancreas-sparing duodenectomies (PSD), 10 pancreaticoduodenectomies (PD), 4 segmental and 3 transduodenal resections. There was no perioperative mortality and 23 patients had 30 complications: 10 patients (22%) with delayed gastric emptying, 8 (17%) with bilio-pancreatic leak, 4 (9%) re-operations. Six patients (4 PD, 1 PSD, 1 Segmental) had carcinoma at operation, 2 known pre-operatively. Post-operative mean follow-up was 45.8 months. Adenomas recurred in 10 patients (4 advanced limb, 6 remnant duodenum); all have been managed endoscopically. Eight patients (5%) died during surveillance, 3 FAP related.

Conclusions: Endoscopic surveillance is valuable for monitoring stage migration and timing of surgical intervention. Complete duodenectomy is the preferred treatment for advanced polyposis.

[3]

Falling Through the Crack: Potential Missed Lynch Syndrome

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Key words: Lynch syndrome, microsatellite instability, Bethesda criteria

Background: The Amsterdam Criteria (AC) and Bethesda Guidelines (BG) are clinical and pathological tools that are used to identify Lynch syndrome (LS) patients. A family history of LS-related cancers, histopathology suggestive of microsatellite instability (MSI-H), or confirmation of MSI-H in a patient with colorectal cancer should prompt an appropriate response by the pathologist and surgeon. This study evaluates the impact of pathology findings and MSI-H status on LS evaluation by the pathologist and surgeon.

Methods: MSI-H tumors were identified from a single institution frozen tissue bank, and MLH-1 methylation was determined by MethyLight quantitative PCR. Clinical information including demographics, histopathology, and personal and family cancer history was recorded. Subsequent recommendations by the pathologist and surgeon for surveillance, genetic testing, counseling, and family risk evaluation were collected from the medical record.

Results: 69 patients with MSI-H tumors were identified between 2000-2007. The median age at resection was 73 years (34-89 years), 48% were female, and 81% of tumors localized to the right colon. 17 (25%) patients were appropriately referred for additional testing including 8 of 9 with MSI-H like histology and 9 of 60 without MSI-H documentation by the pathologist (Table 1). 52 (75%) patients with MSI-H tumors escaped detection by pathologists and surgeons and no additional workup for LS was performed. MSI-H like histology was the most influential factor for further testing, as 89% of those patients had subsequent LS investigation. 34 (49%) of the MSI-H tumors showed no methylation at MLH-1, including 4 patients who met AC, had MSI-H like histology, but had no further LS testing (Table 1).

Conclusions: These data show that MSI-H like histology was the driving force for the evaluation of LS. Histopathology alone failed to identify all potential LS patients. Omission of an adequate familial risk assessment may lead to missed diagnosis of LS when the presence of MSI-H like histology fails to trigger appropriate testing. Our findings underscore the importance of adequate familial risk assessment in the identification of patients with LS and the importance of further testing when MSI-H like histology is present.

Table 1.

	Further investigation	No further investigation	P value
Pathologically suspicious for MSI-H	8 (89%)	1 (11%)	<0.001
Not pathologically suspicious for MSI-H	9 (15%)	51 (85%)	<0.001

[4]

Down-Regulation of the Growth-Suppressor Gene CDK2-AP1 in MSI CRC is Confirmed in Human CRC Tissues and a Mouse Model of MSI CRC

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Key words: microsatellite instability, CDK2-AP1, Mlh1 knockout mouse

Background: We have reported (in this forum and published) that down-regulation of the growth-suppressor gene, cyclin-dependent kinase 2-associated protein 1 (CDK2-AP1) in microsatellite unstable (MSI) human colorectal cancer (CRC) cell lines is associated with increased proliferation and decreased apoptosis in those cell lines. In this report we expand our study of CDK2-AP1 to include a mouse model of MSI CRC as well as human CRC tissues to validate our cell line based observations.

Methods: The DNA mismatch repair (MMR) status of tumors arising from a knockout mouse, generated in our collaborator's laboratory ($Mlh1^{-/-}/APC^{1638N}$), was determined by measuring Mlh1 expression by Western blot and was confirmed by sequencing four mouse microsatellite markers (D9Mit67, D1Mit79, L24372 and U12235). CDK2-AP1 expression in the MSI mouse tumors and adjacent normal mucosa was assessed using RT-PCR and Western blot analysis. A human CRC tissue array containing 43 microsatellite stable (MSS) and 7 MSI CRC tumors was analyzed for CDK2-AP1 expression using standard immunohistochemistry techniques.

Results: All four of the GI tumors harvested from the Mlh1 knockout mouse exhibited the MSI phenotype and had significantly decreased levels of CDK2-AP1 mRNA ($p<0.0001$) and protein. Normal adjacent mucosa from the Mlh1 knockout mouse demonstrated normal CDK2-AP1 expression. In contrast, tumors harvested from a control transgenic APC^{1638N} mouse demonstrated the MSS phenotype and normal CDK2-AP1 expression as measured by mRNA and protein analysis. Furthermore, human CRC tissue array analysis demonstrated 6 of 7 (85.7%) MSI CRC tumors had significantly decreased CDK2-AP1 antibody staining, whereas 0 of 43 MSS CRC tissues demonstrated reduced CDK2-AP1 staining ($p<0.0001$).

Conclusions: These human tissue and mouse model results confirm that down-regulation of CDK2-AP1 is a characteristic of CRC tumors arising from MMR deficiency. These results provide support for the hypothesis that MSI and MSS CRCs are characterized by distinct pathways of malignant transformation and progression and may provide novel molecular-based therapeutic opportunities.

[5]

Early Age Onset Adenomatous Colorectal Polyp Formation Among African Americans is Associated with a Q324H Germline Sequence Variant of the MutY Homolog (MYH) Gene

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Key words: early onset colorectal polyps, MYH Q324H, African Americans

Background: The contribution of germline genomic variation to increased rates of colorectal cancer (CRC) incidence among African Americans remains unknown. Sequence alterations in the MutY human homologue (MYH) gene have been reported to be associated with colorectal cancer predisposition in European populations. This study was designed to test the hypothesis that novel MYH alterations are associated with colorectal adenomatous polyp formation in African Americans.

Methods: One hundred and twenty-three unrelated African Americans adults with histologically confirmed multiple or a single large (>1cm) colorectal polyp(s) were prospectively consented and screened for germline mutation(s) of the MYH gene by direct sequencing. Twenty-nine of 123 members of the test group were ≤50 and 94 were >50 years of age at the time of polyp diagnosis. A control group of 73 healthy African American consented volunteers without history of polyps or CRC were screened for the Q324H variant using a *Bts I* restriction endonuclease.

Results: Sixty-three (51%) of 123 subjects carried the germline MYH Q324H alteration, as indicated by direct sequencing: 50/63 (79%) monoallelic and 13/63 (21%) biallelic. Among controls 11/73 (15%) carried the Q324H alteration: 10 (14%) monoallelic and 1 (1%) biallelic, a statistically significant difference ($p<0.01$). Interestingly, in sub-set analysis the Q324H carrier frequency among subjects diagnosed ≤50 years of age was 86% (25/29); 17/29 (58%) monoallelic and 8/29 (28%) biallelic and 40/94 (42%) among subjects diagnosed after 50; 33/94 (35%) monoallelic and 5/94 (5%) biallelic, a statistically significant difference ($p<0.05$).

Conclusions: These results indicate the MYH germline variant Q324 is associated with adenomatous polyp formation among African Americans especially among individuals diagnosed at or before age 50. For the future

we plan to expand our studies of the Q324 variant to include more diverse populations as well as subjects diagnosed with invasive colorectal cancer.

[6]

Lynch Syndrome Mutation Prevalence in a Large Series of Patients

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Key words: Lynch syndrome, mutation prevalence, genetic testing

Background: Deleterious mutations in the mismatch repair genes *MLH1*, *MSH2* and *MSH6* are known to cause Lynch syndrome (HNPCC). The aim of this study was to measure the prevalence of these gene mutations in groups of patients stratified by personal and family history as reported on the test requisition form of a large commercial laboratory.

Methods: Myriad Genetic Laboratories offers full sequencing and Southern blot of *MLH1* and *MSH2* for detection of mutations causative of Lynch syndrome. The more recent addition of *MSH6* sequencing has enhanced this analysis. For the current study, we report the prevalence of mutations identified in our overall test population and after stratification by personal and family history.

Results: Of 6,826 *MLH1* and *MSH2* gene analyses performed, 916 (13.4%) patients were identified with deleterious mutations. Of these patients, 388 had mutations in the *MLH1* gene, representing 42.4% of all mutation positive patients and 5.7% of the total tested population. Five hundred twenty-eight (528) patients were identified with a deleterious mutation in *MSH2*, accounting for 57.6% of all mutation positive patients and 7.7% of the total tested population. *MSH6* gene sequencing was performed on a total of 1247 patients; 39 (3.1%) patients had deleterious mutations. In patients reporting a personal history of colorectal cancer (CRC) under age 50 and no family history of Lynch syndrome related cancers, the mutation prevalence was 7.2% (22/306). Patients with CRC under 50 who reported a family history of at least one relative with a Lynch syndrome cancer had a mutation prevalence in either *MLH1* or *MSH2* of 26.5% (282/1064). Patients reporting a personal history of more than one Lynch syndrome related cancer and no family history had a mutation prevalence of 8.8% (6/68). In patients reporting a personal history of more than one Lynch syndrome related cancer and a family history of at least one family member with a Lynch syndrome related cancer, the mutation prevalence was 43.5% (161/370). Patients with a personal history of endometrial cancer under the age of 50 who report at least one family member with a Lynch syndrome cancer, had a mutation prevalence of 29.6% (45/152). In patients reporting a personal history of ovarian cancer regardless of their family history (who may or may not have relatives with Lynch syndrome related cancers), we observed a mutation prevalence of 7.4% (16/216).

Conclusions: Patients with a personal and/or family history of Lynch syndrome related cancers are appropriate candidates for genetic testing of the mismatch repair genes.

[7]

Colorectal Cancer in Young Patients: Unexpected Clustering in the Rectosigmoid Colon, Low Frequency of Classic Lynch Syndrome, and Involvement of JC virus

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Background: Colorectal cancer (CRC) occurs rarely in individuals <50 years, and comprises a heterogeneous group including patients with Lynch syndrome, as well as those with other non-familial, sporadic cancers. Little is know about the full spectrum of factors associated with CRC in young people. This study examined the clinical features, microsatellite instability (MSI), DNA MMR protein expression, and JC virus (JCV) expression in patients <50 years old with sporadic, apparently non-familial CRC.

Methods: Paraffin-embedded tissue sections were obtained from 82 patients with CRC, all <50 years old, none of whom came from a familial cluster of CRC. Immunohistochemical (IHC) staining was performed for the hMLH1, hMSH2, hMSH6, and hPMS2 proteins, and for the T-antigen (T-Ag) of JC virus (JCV), an oncogenic virus found in most sporadic CRCs. MSI analyses were performed using a panel of 5 mononucleotide repeat markers, and used consensus criteria for MSI (≥2 mutations=MSI-H).

Results: The mean age of the patient cohort was 34.4 years (range 16-47 years), and 80/82 were ≤40 years old. Three patients had IBD-associated CRCs, but none had a polyposis syndrome. Only 26% (22/78 – 4 without clinical information) of the tumors were located in the proximal colon, while 74% (56/78) were located

distal to the splenic flexure. Unexpectedly, 41% of the tumors were in the rectosigmoid or rectum. Overall, 17% (14/82) of the CRCs were MMR-deficient, based upon loss of expression of MMR proteins at IHC. Unlike classic Lynch syndrome, only 43% (6/14) of these MMR-deficient CRCs (by IHC) demonstrated loss of expression of one of the two major DNA MMR proteins, hMLH1 or hMSH2 (3 each for hMLH1 and hMSH2), together with the associated losses of hPMS2 or hMSH6. Of particular note, 8/14 (57%) of the MMR-deficient CRCs had experienced the exclusive loss of either hPMS2 (n=3) or hMSH6 (n=5), without the respective loss of expression of hMLH1 or hMSH2. While all 9 tumors with hMLH1, hMSH2, or hPMS2 losses were MSI-H, only 2/5 of the CRCs with isolated losses of hMSH6 were MSI-H; in both cases, just 3/5 markers were mutated and in the other three, none were mutated. In all 3 instances of hPMS2 loss, either 4/5 or 5/5 of the markers were mutated. Finally, 79% of the CRCs showed strong nuclear staining for the JCV T-Ag.

Conclusions: We found that 41% of 82 CRCs in young patients occurred in the rectosigmoid region. In this group of non-familial CRCs, 17% had defects in DNA MMR protein expression, and 57% were atypical, with isolated losses of hMSH6 or hPMS2. Some (3/5) of the CRCs with isolated losses of hMSH6 were MSS, and will be missed by approaches that use MSI to screen for Lynch syndrome. A small but important portion (3/82) of these apparently non-familial CRC patients may have germline mutations in hPMS2, which could be very important for appropriate genetic counseling. Finally, the strong expression of JCV T-Ag in the nuclei of most of these tumors implicates this oncogenic virus in the genesis of CRC in young people, including those with DNA MMR defects.

[8]

Long-Term Risk of Pouch Neoplasia After Ileal Pouch Anal Anastomosis for Familial Adenomatous Polyposis

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Key words: familial adenomatous polyposis, ileal pouch, neoplasia risk

Background: Ileal pouch anal anastomosis (IPAA) is a mainstay of surgical prophylaxis for familial adenomatous polyposis (FAP), and is thought to virtually eliminate the risk of cancer. The development of pouch carcinomas has been reported in isolated cases and a number of cross-sectional studies have evaluated the prevalence of pouch adenomas, but the true long-term risk and natural history of ileal pouch neoplasia remain unknown.

Methods: All available pouch endoscopy and associated histology reports for patients with FAP attending for annual surveillance after IPAA at St Mark's Hospital since 1978 were reviewed retrospectively. The incidence, anatomical location and histological characteristics of pouch neoplasms were recorded. Cumulative adenoma-free survival was calculated using Kaplan-Meier survival analysis.

Results: Of 206 patients who underwent IPAA, 140 attended for endoscopic follow-up at this institution. The median adenoma-free survival in the pre-pouch ileum (PPI), pouch body, and anal transitional zone (ATZ) was 10.1, 7.2 and 8.1 years respectively, with 83%, 18% and 41% of patients remaining adenoma-free at 20 years. Adenomas were mildly dysplastic in 88% of PPI, 81% of pouch and 71% of ATZ polyps, with the remainder being predominantly moderately dysplastic. The maximum size of adenoma recorded was 4 mm in the PPI, 40 mm in the pouch and 60 mm in ATZ. There were no instances of progression in the PPI. In the pouch body, 2 patients developed large villous adenomas (>5 mm), and 8 had tubulovillous adenomas, 3 of which were large. In the ATZ, 4 patients developed large tubulovillous adenomas, and one had adenocarcinoma after 19 years. In total, 4 patients required pouch excision for neoplasia.

Conclusions: The majority of FAP patients develop benign neoplasms in the pouch body and ATZ in the long term. Although malignant transformation is rare, regular endoscopic surveillance is mandatory and further research is required to identify risk factors for pouch malignancy in the long term.

Funding: Alexander von Roon is supported by a Royal College of Surgeons of England Research Fellowship.

[9]

Educational Outreach to Individuals at Risk for Hereditary Colon Cancer

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Key words: HNPCC, educational outreach, cancer registry

Background: Colorectal cancer (CRC) accounts for over 150,000 new cancer cases and 52,000 deaths each year in the U.S. About 3% of CRC is attributed to

Lynch syndrome, also called hereditary non-polyposis colon cancer. Individuals with Lynch syndrome have significant lifetime risk of developing CRC, upwards of 80%, as well as risk for other related cancers. Thus, identifying these individuals is important for reducing morbidity and mortality from associated cancers. Genetic counseling and testing for Lynch syndrome are under-utilized in Colorado. The purpose of this project was to educate Coloradans at risk for hereditary CRC about the benefits of cancer risk assessment and to provide assessment via telephone to individuals without access to this service. A secondary aim was to determine the feasibility of utilizing a cancer registry for identifying at-risk individuals and conducting educational outreach.

Methods: At-risk individuals were identified through the Colorado Central Cancer Registry. Cases were recently diagnosed with CRC who met one of the first three criteria of the revised Bethesda criteria. The physician of record was mailed an educational brochure about hereditary CRC, a brief survey and asked to provide consent to contact their patient(s). Cases were mailed the brochure and a one-page survey to elicit reactions to being contacted via the registry. Follow-up surveys were mailed to cases about 4 months after the initial mailing.

Results: In total, 575 cases and 412 physicians were identified by the registry. About 40% of physicians (169) representing 226 patients provided consent to contact patients. Among physicians who completed the survey, the majority felt the educational information was clearly presented and useful. Most (77%) reported they currently discuss cancer genetics with their patients and 90% felt the registry should provide this information to at-risk patients, with (30%) or without (60%) physician consent. Forty-three of the 181 cases contacted completed the initial survey (23%). Cases were generally glad to have received the information and wanted to know more. Only 4 cases reported concern or worry in response to the information. The majority of cases agreed the registry should send this information; however most preferred that their physicians be consented first. At follow-up, 20 cases reported having had risk assessment in the past 4 months or had intentions to have risk assessment, and about 45% reported having discussed risk assessment with someone. No cases called the toll-free line for genetic information; thus no referrals were made.

Conclusions: The response from physicians and cases regarding both the content of the materials and the mode of delivery was positive, suggesting that targeted outreach using the cancer registry, in combination with physician notification, may be a viable approach to disseminating genetic information. A sizeable proportion of cases either sought risk assessment or discussed it with others, suggesting that mail-based outreach may be effective in increasing uptake of information and/or genetic services. The lack of calls to the information line may reflect patients' preference to confer first with providers and/or persons of trust regarding these issues. Uptake for telephone risk assessment may be improved after establishing clinical relationships with patients.

Funding: This project was funded by the Mountain States Genetics Collaborative.

[10]

The Revised Bethesda Guidelines: Extent of Utilization in a University Hospital Medical Center with a Cancer Genetics Program

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Key words: revised Bethesda guidelines, HNPCC, microsatellite instability

Background: In 1996, the National Cancer Institute hosted an international workshop to define a set of clinicopathologic criteria to help identify patients with colorectal cancer who should be offered microsatellite instability (MSI) testing due to an increased risk for hereditary nonpolyposis colorectal cancer (HNPCC). These criteria were further modified in 2004 and became known as the revised Bethesda guidelines. In our quality assurance study, we aimed to retrospectively evaluate, over the course of one year, the percentage of patients diagnosed and treated for HNPCC-associated tumors who met revised Bethesda criteria, making them eligible for MSI testing, who were referred for genetic counseling services within our institution. This retrospective study identified a number of barriers, both internal and external, which hindered the identification of individuals with HNPCC, thus limiting the ability to appropriately manage these high risk families.

Methods: All HNPCC-associated tumors diagnosed between January 1, 2004 and December 31, 2004 were identified by accessing CoPath, an internal database. Both the Tumor Registry and patients' electronic medical records were then accessed to gather all relevant family history information. The revised Bethesda criteria were applied to each case to ascertain which patients fulfilled at least one criterion that would warrant MSI testing. This list was then cross-referenced with the database of patients referred for genetic counseling within our institution.

Results: A total of 380 HNPCC-associated tumors were diagnosed at our institution between January 1 and

December 31, 2004 of which 41 (10.7%) met at least one of the revised Bethesda criteria. Eight (19.5%) of these patients were referred for cancer genetic counseling of which 2 (25%) were seen by a genetics professional. Ultimately, only 4.9% of eligible patients who met revised Bethesda criteria for MSI testing in 2004 at our institution were seen for genetic counseling. Of importance, it was not possible to determine for 145 HNPCC-associated tumors (38%) whether their personal and family history met at least one of the revised Bethesda criteria.

Conclusions: Our study highlights several barriers, both within and external to our institution, regarding the application of the revised Bethesda criteria to identify at-risk patients with HNPCC-related tumors. These barriers include the lack of detailed family histories captured by health care providers and the failure of pathologists to routinely characterize MSI-H histology in colorectal cancer specimens. Patient compliance represents another barrier identified in part due to limited knowledge regarding the potential benefits of genetic screening and testing for HNPCC, reservations regarding the sensitive nature of information gleaned from genetic testing, and insurance coverage issues due to the lack of recognition of board certified masters and doctoral trained genetics professionals by major insurers.

[11]

The Impact of Genetic Counseling for Attenuated Familial Adenomatous Polyposis

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Key words: attenuated familial adenomatous polyposis, genetic counseling, risk perception

Background: Families with attenuated familial adenomatous polyposis (AFAP) lack the dramatic presentation of hundreds of colon polyps which brings families with classic polyposis to medical attention. The minimal polyp burden, later age of onset, and fewer relatives with cancer may prohibit recognition of a genetic syndrome by clinicians and lead to underestimation of risk by family members. Therefore families with AFAP may have unique genetic counseling needs. In this study we evaluated the impact of genetic counseling on distress, cancer worry, risk perception, and knowledge in members of a large AFAP kindred previously described by Burt et al. in 2005.

Methods: Members of an AFAP kindred who were at 50% risk for having inherited a familial *APC* mutation were offered the opportunity for free genetic counseling and testing. Standardized measures including the Center for Epidemiologic Studies Depression Scale and Impact of Events Scale, along with true/false, cancer worry and risk perception questions developed based on previously validated tools were used. Differences between pre- and post-counseling were assessed using the Wilcoxon signed rank test.

Results: Forty-four of 99 subjects enrolled in the genetic testing study completed pre- and post-counseling written questionnaires. Eighty percent of participants reported education past high school. Following genetic counseling, participants were found to have significantly lower levels of depressive symptoms ($p \leq 0.0001$). Twenty-four percent had an increase in cancer specific worry, but over the entire group of participants the change was not significantly different pre- and post-counseling ($p=0.4$). Prior to genetic counseling, participants underestimated their risk of having a genetic mutation. After counseling 42% increased their risk estimate, resulting in an accurate mean of approximately 50% ($p=0.0065$). Improvements in knowledge were also observed ($p=0.058$).

Conclusions: These data are from a well studied family in which members were notified that there was a known genetic mutation prior to enrolling. Despite this, most individuals underestimated their risk prior to counseling. It is likely that other AFAP families that have not experienced intensive outreach interventions may be less aware of their risks. Genetic counseling may be an effective approach for improving the accuracy of risk perception and knowledge about hereditary cancer. These data also suggest that through the genetic counseling process, individuals can be informed of their cancer and genetic risks without inducing increased distress or depression. However, in order for individuals at risk for AFAP to access genetic services, further education is needed to ensure that clinicians are appropriately identifying and referring these families.

[12]

The Frequency of the Revised Bethesda Guidelines and Additional Criteria in a Large Population Database

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Key words: Lynch syndrome, revised Bethesda guidelines, endometrial cancer

Background: The revised Bethesda guidelines (BG) were developed to identify individuals who are appropriate candidates for tumor testing using microsatellite instability (MSI) analysis. Except for criterion II, the BG currently require a diagnosis of colorectal cancer (CRC) in individuals being evaluated for Lynch syndrome. It has also been suggested that individuals with endometrial cancer meeting certain criteria be included in the BG due to the high lifetime risk associated with Lynch syndrome. Here we determine the prevalence of individuals in a large population database who meet the BG using both colorectal and endometrial cancer as the indication for evaluation.

Table 1. Revised Bethesda guidelines (Umar et al. 2004)

Requires at least one of the following in an individual being evaluated:
(I) Colorectal cancer diagnosed prior to age 50
(II) Presence of two primary Lynch syndrome-associated tumors*
(III) Colorectal cancer diagnosed less than 60 years of age with MSI-high histology
(IV) Colorectal cancer and one or more first degree relatives with a Lynch syndrome-associated tumor*, with at least one of the cancers being diagnosed under age 50 years
(V) Colorectal cancer and two or more first or second degree relatives with a Lynch syndrome-associated tumor*, regardless of age

*colorectal, endometrial, stomach, ovarian, pancreas, sebaceous gland, ureter, renal pelvis, and biliary tract cancer, keratocanthoma, glioblastoma, and carcinoma of the small bowel

Methods: Using the Utah Population Database (UPDB), which links Utah family histories to Utah cancer records, we determined the number of individuals that met BG I, II, IV or V. BG III was not evaluated due to lack of available records necessary to determine this criterion. Individuals meeting BG II are not required to have a diagnosis of colorectal or endometrial cancer, as any two Lynch syndrome-associated tumors meet the criteria. We also evaluated the frequency of BG I, IV, and V using endometrial cancer as the indication for testing.

Results: Of the 25,806 CRCs in the UPDB, 2346 (9.1%) met BG I, 675 (2.6%) met BG IV, and 1,781 (6.9%) met BG V. There were 7754 endometrial cancers in the UPDB and when these cancers were included in the evaluation, 3670 (10.9%) individuals met BG I, 1,054 (3.1%) met BG IV, and 3,226 (9.6%) met BG V. Of the 49,038 total cases of Lynch syndrome-associated tumors in the UPDB, 1480 (3%) met BG II. There were 5,483 individuals that met at least one BG, and an additional 2,679 individuals met criteria when endometrial cancer was included.

Conclusions: The UPDB provides a unique resource to evaluate the frequency of probands meeting current and proposed guidelines for evaluation of Lynch syndrome. Including both endometrial and colorectal cancer as indications for testing resulted in 8,162 individuals meeting criteria, compared to 5,483 cases when endometrial cancer was not included in BG I, IV or V.

[13]

BRAF Mutations in Hereditary Non-Polyposis Colorectal Cancer and Bethesda Criteria Patients: a Pilot Study

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Key words: *BRAF*, microsatellite instability, Bethesda criteria

Background: Hereditary non-polyposis colorectal cancer (HNPCC) syndrome is clinically defined by the Amsterdam criteria. However, some patients do not match these criteria exactly and therefore are classified as suspected HNPCC, according to Bethesda guidelines. At a molecular level, HNPCC patients are characterized by "germ-line" mutations in mismatch repair (MMR) genes leading to microsatellite instability (MSI). MMR genes can be altered at a somatic level also in up to 15% of sporadic colorectal cancers (CRC).

Interestingly, *BRAF* mutations occur more frequently in MSI than in microsatellite stable (MSS) sporadic CRCs and particularly segregate with MLH1 promoter hypermethylation. Notably, no *BRAF* mutations have been identified in HNPCC patients with known germ-line MMR gene mutation. However, little is known about *BRAF* status in suspected-HNPCC patients, who, by definition, have a high risk of carrying mutations in MMR genes. Since *BRAF* mutation and HNPCC are mutually exclusive, our aim was to verify whether the presence of *BRAF* mutation may help clinicians in the identification of suspected HNPCC patients with MSI profile avoiding the assessment of germ-line MMR mutation.

Methods: We selected 120 CRCs (Register of Hereditary Colorectal Tumours) including 27 HNPCC patients fulfilling Amsterdam criteria and carrying germ-line MMR gene mutation, 37 suspected HNPCC patients

according to Bethesda guidelines and 56 sporadic patients. Microsatellite status and *BRAF* mutations were investigated according to standard procedures already published.

Results: MSI was found in 40 out of 64 (62.5%) HNPCC and suspected-HNPCC cases. No *BRAF* mutations were found in 27 HNPCC patients. In the suspected HNPCC patient group, only 2 out of 37 (5%) tumours showed *BRAF* mutations, including the classical V600E in a MSI case and the not frequent, but already reported, N580S change in a MSS case. As comparison, MSI was found in 10 out of 56 (18%) sporadic cases and the classical BRAF V600E mutation was detected in 2 out of 10 (20%) MSI and in 1 out of 46 (2%) MSS sporadic cases.

Conclusions: *BRAF* mutations are associated with MSI sporadic CRCs, and absent in HNPCC patients, thus confirming previous reports. Moreover, *BRAF* is rarely mutated in suspected HNPCC patients (Bethesda guidelines). Therefore, in suspected HNPCC the *BRAF* mutation testing seems to have a modest impact on the exclusion of patients from the characterization of germline MMR gene mutations.

[14]

Rapid Activation of Hereditary Colorectal Cancer Counseling Program

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Background: Beth Israel Deaconess Medical Center (BIDMC) has an established Genetics and Cancer Prevention Program that had low level activity in the area of hereditary colon and rectal cancer.

Methods: We formed a small working group of physicians (gastroenterology, colorectal surgery, pathology and medical oncology), genetic counselors and hospital administrators to create a Hereditary Colorectal Cancer Program (HCRCP) with defined management pathways for patients and family members at risk for, or diagnosed with, inherited colorectal cancer. We added a second gastroenterologist (January 2007). We added reflex microsatellite instability testing (msi) for all colon cancer specimens in patients age 50 and under in our institution. We upgraded and updated our computerized data collection system (Progeny). We prospectively tracked referrals to the Program and compared them to historical volumes.

Results: Our program-building work was initiated at the beginning of fiscal year 2007 (October 2006) with an immediate increase in referral volume (Table 1). Reasons for referral included: patients with colon cancer, family history of colon cancer, family history of hereditary non-polyposis cancer (HNPCC) and polyps. There was an increase in rate of referral for family cancer syndromes and polyps compared to historical volumes. We saw a significant increase in referrals from gastroenterology, medicine and hematology-oncology.

Table 1. Referrals to BIDMC-HCRCP by fiscal year

FY	Q1-2	Q3-4
FY 04	4	7
FY 05	3	4
FY 06	0	0
FY 07*	16	30 (annualized)

**15 thru June 2007*

Conclusions: With a small group organizational effort to create basic structure, but without formal program promotion, we significantly increased referrals to and utilization of our Hereditary Colon and Rectal Cancer Program. In particular, medical specialities demonstrated increased knowledge and awareness of hereditary cancer syndromes via their referrals. Next steps at our institution include program promotion and research initiatives.

[15]

Cyclooxygenase-2 and Platelet-Derived Growth Factor Receptors as Potential Targets in Treating Aggressive Fibromatosis

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Key words: aggressive fibromatosis, RTK activation profile, COX-2, PDGFRA, PDGFRB

Background: To explore the molecular bases of potential new pharmacological targets in aggressive fibromatosis (AF) (desmoid tumour).

Methods: Tumour specimens from 14 patients surgically treated for AF, 6 familial adenomatous polyposis (FAP) and 8 sporadic cases, analyzed for APC and CTNNB1 (β-catenin) mutations were further investigated for β-catenin, COX-2, PDGFRA/PDGFRB, their cognate ligands (PDGFA and PDGFB) and KIT using a comprehensive immunohistochemical, biochemical, molecular and cytogenetic approach.

Results: No CTNNB1 (β-catenin) mutations were found in the FAP patients, but previously reported activating mutations were found in 6 of the 8 sporadic patients. All of the cases carrying an altered WNT pathway showed nuclear and cytoplasmic immunoreactivity for β-catenin, whereas β-catenin expression was restricted to the cytoplasm in the sporadic patients lacking CTNNB1 mutations. COX-2 protein and mRNA overexpression was detected in all 14 cases, together with the expression and phosphorylation of PDGFRA and PDGFRB, which in turn paralleled the presence of their cognate ligands. No PDGFRB mutations were found. The results are consistent with PDGFRA and PDGFRB activation sustained by an autocrine/paracrine loop.

Conclusions: AF is characterized by WNT-oncogene pathway alterations triggering COX-2-mediated constitutive co-activation of PDGFRA and PDGFRB and may benefit from combined non-steroidal anti-inflammatory drugs (NSAIDs) + tyrosine kinase inhibitor treatment.

[16]

Long-Term Follow up of Subjects to Assess Changes in the Family History of Colorectal Cancer and to Identify New Family History Risks and New Hereditary Colorectal Cancer Syndromes

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Key words: family history, colorectal cancer, surveillance recommendations

Background: Family history of colorectal cancer is an important risk factor for the disease. The strength of family history determines the intensity of colorectal cancer screening or surveillance. Few studies have investigated changes in familial risk for colorectal cancer and the impact of the change on the frequency of screening or surveillance recommendations.

To determine if a family history of colorectal neoplasia changes after 10 years and whether the change affects screening or surveillance recommendations.

Methods: The study cohort was incepted in 1991 during a family history-based, public colorectal cancer screening program. The program offered a free risk assessment to callers and free flexible sigmoidoscopy to individuals with one first or second degree relative with colorectal cancer. 1440 people participated in the risk assessment and they were categorized into three groups. Group 0 (no family history), Group I (one first or second degree relative affected with colorectal cancer), and Group II (more than one first or second degree relative affected). In 2001, responders were contacted by phone to update their family history of colorectal neoplasia. Those not contactable by phone were sent a questionnaire.

Results: Responses were obtained from 30% in Group 0 (122/407), 39% in Group I (132/336), and 15% in Group II (104/697) (Table 1).

Conclusions: Family history of colorectal cancer increased over 10 years in 26% of families. The change appeared to be stronger in originally weak family histories. These changes resulted in a reassignment of risk and change in screening or surveillance recommendations in 58% (54/93) of families, as well as identified new families with hereditary colorectal cancer syndromes. These data underscore the crucial necessity of updating family history of colorectal cancer in order to provide screening or surveillance recommendations that reflect current risk of the disease.

Table 1. Change in family history of CRC and screening/surveillance recommendations over 10 years

Group	N responders in 2001	N (%) with new FHx	% FHx change based on CRC	N with new HNPCC based on AM-I	N with change in Scr/Sur Recs	N with no change in Scr/Sur Recs
0	122	33 (27%)	45%	0	22	11
I	132	40 (30%)	48%	1	30	10
II	104	20 (19%)	31%	2	2	18
Total	358	93 (26%)	42%	3	54	39

N – number of participants; FHx – family history; CRC – colorectal cancer; AM-I – Amsterdam I Criteria for Hereditary Nonpolyposis Colorectal Cancer; Scr/Sur – screening/surveillance; Recs – recommendations

[17]

HNPCC and Endometrioid Cancer: Case Report and Review of the Literature

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Key words: endometrioid cancer, endometriosis associated intestinal tumors, HNPCC

Background: Hereditary nonpolyposis colorectal cancer (HNPCC) is a disorder of DNA mismatch repair that has autosomal dominant inheritance and leads to increased risk for colon and endometrial cancer, amongst others. Independent of HNPCC, endometrioid cancer has been reported to arise in foci of endometriosis with endometriosis-associated intestinal tumor (EAIT) being a rare variant.

Methods: We report an HNPCC patient with endometrioid cancer despite a hysterectomy twelve years earlier. We also present a review of the English literature through a Medline search from 1970-2007 of endometrioid cancer in HNPCC and EAIT.

Results: An otherwise healthy 54-year-old female (family history fulfilled Amsterdam II criteria for HNPCC) status post hysterectomy and bilateral oopherectomy for endometriosis (age 42) and status post right colectomy for a stage I colon cancer (age 49) presented with complaints of pelvic pain. A 1.5 cm mass was found on CT between the rectum and vaginal cuff. Colonoscopy was normal. She underwent a margin negative, low anterior resection with en bloc resection of the pelvic mass and vaginal cuff. Pathology showed a serous adenocarcinoma arising from a focus of endometriosis with transmural involvement of the colon. One of twelve lymph nodes was positive for cancer and she underwent adjuvant chemotherapy with carboplatin and taxol. She presented within months with tumor seeding of the wound, and repeat CT scan showed a recurrence of her pelvic mass. External beam radiation to the wound and pelvis, followed by repeat surgical excision (abdominoperineal resection) with negative margins, failed to prevent recurrence. Nineteen months after her original presentation, the patient entered hospice after failing further salvage chemotherapy. Microsatellite instability (MSI) and immunohistochemistry testing of her colon cancer showed it to be MSI-high with a loss of MSH2. Mutational analysis is pending. Literature review shows 19 cases of EAIT within the rectosigmoid colon. There are no reported cases of endometrioid cancer associated with HNPCC. Hyper-estrogenism is an identified risk factor for endometrioid cancer. Endometrioid cancer is usually low grade and confined to its site of origin, so complete surgical excision is recommended. There is an 82% overall five year survival rate with survival for disseminated disease being only 12.5% at five years. Chemotherapy is of unclear benefit.

Conclusions: Endometrioid cancer is rare and not previously reported in HNPCC. This malignancy must be considered in patients with HNPCC and a history of endometriosis despite previous hysterectomy. Outcomes of disseminated endometrioid cancers and EAIT are poor and require an aggressive multidisciplinary approach.

[18]

Fate or Family: Do African American Men Have a Higher Risk of Colorectal Cancer Due to Family History?

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Background: Colorectal cancer is the third most common cancer found in men and women in the United States. Over the past 15 years the death rate from colorectal cancer has decreased due to fewer cases as a reflection of improved colorectal cancer screening. African Americans have the highest number of colorectal cancer cases and the highest death rates from colorectal cancer of all racial groups in the United States. The reason for this is not yet known and should be explored.

Methods: Participants were recruited at a minority health fair where African American men were asked to complete a validated family history scoring system which was previously published by our group. The program was designed where family history was entered by answering a series of questions, including the relationships of affected relatives to the person being screened, and their age at diagnosis. The program calculated the Family History Score. No identifying information was collected. Recommendations for surveillance were given depending on the risk level assigned. Patients who score 0 are of average risk and are not significantly different than the average population. Patients with scores <7 are considered at low familial risk of cancers or advanced adenomas while scores >8 signify high risk. Scores >11 are suggestive of a hereditary colorectal cancer syndrome. This study was approved by the Institutional Review Board at the Cleveland Clinic.

Results: 157 African American men participated. The scores are shown in the Table 1.

Table 1.

Score	Risk level	N
0	average population	130 (83%)
1-7	low increased	23 (15%)
8-10	high increased	3 (2%)
>10	likely HNPCC	1 (1%)

18% of African American men had an increased risk of colorectal cancer based on family history. One patient with likely HNPCC had a score of 15.

Conclusions: Using the Family History Scoring System, African American men are at a significantly higher risk of colorectal cancer due to family history. These findings suggest that family history may be one of the contributing factors for an increased morbidity rate in African Americans. A comprehensive pedigree should be taken when interviewing minorities during patient visits in an effort to educate those at risk and provide appropriate screening.

[19]

Recruitment of Large Colorectal Cancer Families Through a Population-Based Cancer Registry

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Key words: family expansion, recruitment, population-based cancer registry

Background: This study demonstrates the feasibility of using a statewide cancer registry to contact cancer probands and expand the family for clinical and genetic study. The objective was to identify large high-risk colorectal cancer (CRC) families from the Utah Population Database (UPDB) and to expand and recruit these large families from CRC probands contacted through the Utah Cancer Registry (UCR). UPDB is a genealogic resource containing over 5 million individual records of people who had a significant life event (birth, death, childbirth) in Utah or who are ancestral to current members of the Utah population. UPDB is also linked to the state-wide UCR.

Methods: Families for study were selected from UPDB as having a significant p-value for familial aggregation of CRC ($p<0.05$) and an increased familial standardized incidence ratio (FSIR, ratio of observed to expected) for CRC. CRC cases in the families were contacted by UCR through a letter asking them, or their next-of-kin (NOK), permission to be contacted by the study. The study then contacted interested individuals and expanded the kindred through family referral. Referrals were made both verbally and through a written family contact form.

Results: UCR sent letters to 404 CRC cases diagnosed from 1966 to 2003 for referral to research. Contact was made on 261 cases (65%) by contacting the probands (n=210) or their NOK (n=51); the remaining 143 cases were unavailable. Consent was obtained on 76% (198 cases) of those contacted. The NOK were more likely to consent (94%) vs. the probands (72%). We report on family expansion from UCR contact (n=32) obtained for six large kindreds (of 78 requests submitted, 9 declined contact or were unavailable and 37 are still pending). The average number of CRC cases per family was 11.3 (median=11; range=4 to 17). In total, the study contacted 8 cases and 14 NOK from the six kindreds. To date, 1 case and 4 NOK failed to result in family expansion, whereas 7 cases and 10 NOK resulted in 78 and 68 family members enrolled, respectively. Ten cases/NOK have not yet been contacted. Recruitment for three families is now complete and the numbers of participants (n=44, n=40, and n=24) is sufficiently large for genetic analysis.

Conclusions: We report implementation of a successful model for recruitment from individual CRC cases contacted through a statewide cancer registry. Although UPDB is a unique resource, this model can be generally applied to smaller families expanded from individual cancer cases identified through other population-based cancer registries. Of those cases and NOK successfully contacted by UCR, the majority provided consent to be contacted by the study and referral to their family members for expansion.

[20]

Darwin Meets Familial Adenomatous Polyposis: the Evolution of Prophylactic Surgery in Response to Two Major Developments in Technique

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Key words: familial adenomatous polyposis, laparoscopy, ileo-pouch anal anastomosis

Background: Colectomy with ileo-rectal anastomosis (IRA) for familial adenomatous polyposis (FAP) was the standard from 1919 until the development of the ileo-pouch anal anastomosis (IPAA). Ten years later, laparoscopic colectomy began. The aim of this study was to review all the index surgeries for FAP performed at our institution assessing the effect of changes in surgical techniques.

Methods: All index FAP surgeries performed at this institution were reviewed. The procedures were stratified by date using 1983 (first IPAA) and 1992 (first laparoscopic colectomy).

Results: 395 patients were included; 51.8% were men. Follow-up ranged from 1 to 540 months, (median 48 months). Before 1983, 97% (66/68) of surgeries were IRA. The rate of a completion proctectomy was 46% (31/66). After 1983 60% of surgeries were IRA and 38% were IPAA. 69% with severe phenotype (>1000 polyps) had IPAA. Between 1992 and 2006, 39% (70 IRA/17 IPAA) of the surgeries were laparoscopic, with 4.6% (4/87) conversion rate. From 126 IPAA surgeries, 32% (40/126) were one-stage and mucosectomy was performed in 23% (29/126). In those patients who had surgery and a genetic test, the mutation was below codon 1068 in 85% (29/34) of the IRA group and above codon 1287 in 86% (6/7) of the IPAA group.

Conclusions: Colon surgery for FAP has evolved as advances in surgical technique allow more effective polyp control while preserving quality of life. Current strategy uses polyposis severity to decide which surgery and laparoscopy to minimize morbidity.

[21]

Prospective Analysis of Computed Tomographic Colonography in the Evaluation of Familial Adenomatous Polyposis

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Background: Patients with familial adenomatous polyposis (FAP) need prophylactic colectomy or proctocolectomy to prevent colorectal cancer. The type, techniques and timing of surgery are determined by various aspects of their phenotype and genotype, including the severity of the colorectal polyposis and the possibility of intra-abdominal desmoid tumors. We performed this study to see if computed tomographic colonography (CTC) would be an efficient and reliable way of assessing the status of the colon and the abdominal contents in general, thus facilitating surgical strategy in patients coming to surgery with FAP.

Methods: Patients 14 years old or older having their index colonic surgery for FAP were eligible. After informed consent patients underwent CTC within 24 hours of the surgery. Results from CTC were recorded on a standard datasheet and were compared with findings at colonoscopy, pathologic inspection of the colon removed at surgery, perioperative EGD, and the results of intraoperative exploration of the abdomen.

Results: 7 patients were enrolled, 6 male and 1 female. Their mean age at diagnosis of FAP was 28 years and at surgery was 30 years. No patient had a cancer clinically, on colonoscopy or on CTC. The polyposis burden is shown in the Table 1. No patient had intra-abdominal or abdominal wall desmoids. 3 patients had extracolonic findings seen only on CTC while in 0 patients these were confirmed at laparotomy.

Table 1.

	Attenuated FAP	Mild polyposis	Severe polyposis	Colorectal cancer
Optical colonoscopy	1	2	4	0
CTC	1	3	3	0
Pathology	1	1	5	0

Conclusions: This pilot study shows that CTC is a valuable tool for the assessment of patients coming to surgery with FAP, allowing assessment of both the colon and the abdominal contents and potentially affecting surgical strategy.

[22]

Ileal Polyp Formation Above Ileo-Rectal Anastomosis in Familial Adenomatous Polyposis

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Key words: familial adenomatous polyposis, extra-colonic, ileum

Background: Macroscopic ileal polyps are very rare in familial adenomatous polyposis prior to colectomy. Increasingly, it is recognised that the ileoanal pouch can develop adenomas following restorative proctocolectomy. This is the first retrospective study of a similar pheno-

menon occurring in the ileum above the ileorectal anastomosis (IRA), in a large cohort of patients.

Methods: 202 FAP patients undergoing 6 monthly rectal surveillance following IRA were identified at St Mark's Hospital, and endoscopy and pathology reports were reviewed.

Results: There were 169 patients without ileal polyps, and 33 with adenomatous polyps noted in the ileum above the rectal anastomosis. Average time since IRA was 13.2 and 20 years respectively ($p<0.05$). 80% of polyps were mildly dysplastic, and the remaining polyps had moderate dysplasia. 86% were under 5 mm in size, but the largest polyp noted was 15 mm, which was moderately dysplastic, and removed endoscopically. 6 patients had >10 adenomas. Notwithstanding the small sample size, there was no obvious genotype-phenotype correlation, nor correlation with rectal polyp burden.

Conclusions: These findings support the hypothesis that surgery triggers tumorigenesis in the FAP small bowel, possibly in a time-dependent manner, due to exposure to an altered environmental milieu. While there are no reports of ileal cancers in the setting of an IRA, the finding of larger dysplastic lesions suggests that progression to cancer is theoretically possible. Thus the ileum proximal to the IRA should be examined as part of the surveillance of the rectum. Further study of small bowel tumorigenesis in FAP may pinpoint environmental triggers for adenoma formation.

Funding: Olivia Will is supported by the St Mark's Polyposis Registry, and Cancer Research UK.

[23]

The Importance of Continued Follow-Up and Cancer Genetic Evaluation as Illustrated in a Family with Gardner's Syndrome

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Background: The scientific community continues to rapidly discover cancer predisposition genes as well as more accurately define the phenotypic features of these syndromes. These advancements assist in the molecular identification of high-risk families and provide more effective and individualized medical management. Therefore, continued follow-up becomes critical and is exemplified by Family A, a case that had been lost to follow-up for almost 40 years. Family A was first reported in the literature in 1955 by Weiner and Cooper, presenting with a dominant inheritance pattern of osteomatosis, multiple soft tissue tumors and colorectal adenomatosis polyposis consistent with several families previously reported by Gardner et al. between 1950 and 1953. The clinical diagnosis of Gardner syndrome was then established. In 1969 Coli et al. further described the phenotypic manifestations of Family A's second and third generations, after which the family was largely lost to follow-up until two second generation members presented to our high-risk clinic.

Methods: An updated family history was collected and compared to that previously reported in the literature. As a result of the gynecologic tumors found, a literature search was conducted evaluating the frequency of this tumor type as an associated feature of FAP/Gardner syndrome.

Results: The four relatives counseled in our high-risk clinic were educated regarding the appropriate medical follow-up, as well as the fact that molecular testing for Gardner syndrome is now available. The only remaining relative clinically affected with Gardner syndrome is awaiting his APC results. Due to the presence of male breast cancer, female breast cancer and ovarian cancer BRCA gene analysis was performed in two affected women, which failed to identify a mutation. One of these women was also found negative for APC and MYH mutations. An updated pedigree revealed new cases of breast, ovarian and primary peritioneal cancers in the second and third generations. Additional tumors were also reported which include a 39-year-old woman diagnosed with a glioblastoma and a woman diagnosed with a benign orbital mass and thyroid tumor.

Conclusions: The presence of multiple newly reported cases of breast and ovarian cancers within Family A raises concern for either two independent cancer predisposition syndromes, hereditary breast and ovarian cancer susceptibility syndrome (HBOCS) and familial adenomatous polyposis (FAP) or the possibility for the rare occurrence of gynecologic cancers within a Gardner syndrome family. The latter has been previously reported in a small case series and may be supported by the lack of identifiable mutations on BRCA sequencing. The dynamic changes in this pedigree underscore the importance of continued follow-up for cancer families in which the underlying etiology is unknown. Such families should be followed in cancer genetics clinics to update family histories, ensure that appropriate screening regimens are followed, and to offer genetic testing if the appropriate analysis becomes available.

[24]

Novel Mutation in MSH6 and Adrenocortical Carcinoma

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Key words: Lynch syndrome, MSH6, adrenocortical carcinoma

Background: Definition of the spectrum of cancers associated with germline mutations in the mismatch repair (MMR) genes is important for the accurate counseling and management of high-risk families. Lynch syndrome is associated with tumors of the colon, endometrium, ovary and stomach. Tumors of other organs, including the small bowel, urinary tract, gall bladder, bile ducts and brain are rare, occurring in less than 5% of individuals with Lynch syndrome. Rare cancers such as adrenocortical carcinoma are not considered part of the Lynch syndrome tumor spectrum. Case reports of adrenocortical carcinoma in families with inherited *MSH2* mutations provide evidence both for and against the association of this rare cancer with MMR defects.

Methods: A 49-year-old female was referred for hereditary cancer evaluation after a diagnosis of moderately differentiated and partially mucinous adenocarcinoma of the cecum. The patient had a history of thyroid cancer of unknown histological type at age 25 years. Family history was significant for endometrial carcinoma in the patient's mother at 40 years of age. Pre-screening for Lynch syndrome included microsatellite instability (MSI) testing of the tumor and immunohistochemical (IHC) staining for MLH1, MSH2 and MSH6.

Results: Results were MSI high and absent staining for MSH6 protein with positive staining for MLH1 and MSH2 proteins. Germline testing revealed the novel frame shift mutation c.3312insT in exon 5 of MSH6. In the course of counseling and management, our patient's 53-year-old sister was found to have a large abdominal mass. A colonoscopy was negative and a hysterectomy had been performed in the past for benign disease. Needle biopsy of the mass revealed a poorly differentiated carcinoma with IHC staining consistent with adrenocortical carcinoma. IHC staining for MSH6 protein was negative and the patient was found to carry the c.3321insT mutation. MSI and loss of heterozygosity (LOH) studies have not been performed to date.

Conclusions: Our case provides further evidence that an underlying MMR defect may be causative in cases of rare cancers diagnosed in Lynch syndrome families. In evaluating families for possible Lynch syndrome, it is important to consider all cancers, as defects in the cellular DNA repair system may contribute to the etiology of a wider range of tumors than previously thought.

[25]

Lynch Syndrome Mutation Prevalence in a Large Series of Patients

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Key words: Lynch syndrome, mutation prevalence, genetic testing

Background: Deleterious mutations in the mismatch repair genes *MLH1*, *MSH2* and *MSH6* are known to cause Lynch syndrome (HNPCC). The aim of this study was to measure the prevalence of these gene mutations in groups of patients stratified by personal and family history as reported on the test requisition form of a large commercial laboratory.

Methods: Myriad Genetic Laboratories offers full sequencing and Southern blot of *MLH1* and *MSH2* for detection of mutations causative of Lynch syndrome. The more recent addition of *MSH6* sequencing has enhanced this analysis. For the current study, we report the prevalence of mutations identified in our overall test population and after stratification by personal and family history.

Results: Of 6,826 *MLH1* and *MSH2* gene analyses performed, 916 (13.4%) patients were identified with deleterious mutations. Of these patients, 388 had mutations in the *MLH1* gene, representing 42.4% of all mutation-positive patients and 5.7% of the total tested population. Five hundred twenty-eight (528) patients were identified with a deleterious mutation in *MSH2*, accounting for 57.6% of all mutation positive patients and 7.7% of the total tested population. *MSH6* gene sequencing was performed on a total of 1247 patients; 39 (3.1%) patients had deleterious mutations. In patients reporting a personal history of colorectal cancer (CRC) under age 50 and no family history of Lynch syndrome related cancers, the mutation prevalence was 7.2% (22/306). Patients with CRC under 50 who reported a family history of at least one relative with a Lynch syndrome cancer had a mutation prevalence in either *MLH1* or *MSH2* of 26.5% (282/1064). Patients reporting a personal history of more than one Lynch syndrome

related cancer and no family history had a mutation prevalence of 8.8% (6/68). In patients reporting a personal history of more than one Lynch syndrome related cancer and a family history of at least one family member with a Lynch syndrome related cancer, the mutation prevalence was 43.5% (161/370). Patients with a personal history of endometrial cancer under the age of 50 who report at least one family member with a Lynch syndrome cancer, had a mutation prevalence of 29.6% (45/152). In patients reporting a personal history of ovarian cancer regardless of their family history (who may or may not have relatives with Lynch syndrome related cancers), we observed a mutation prevalence of 7.4% (16/216).

Conclusion: Patients with a personal and/or family history of Lynch syndrome related cancers are appropriate candidates for genetic testing of the mismatch repair genes.

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Survey of Cancers and Genotype-Phenotype Correlations in the Lynch Syndrome Population at U.T. M.D. Andreson Cancer Center

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Background: Hereditary non-polyposis colorectal cancer (HNPCC/Lynch syndrome) is an autosomal dominant cancer predisposition syndrome caused by germline mutations in mismatch repair (MMR) genes – mainly hMLH1, hMSH2, hMSH6, and hPMS2. The purpose of this study was to examine the prevalence of cancers and evaluate genotype-phenotype correlations in patients from a heterogeneous North American patient population.

Methods: A retrospective medical record review was performed on patients who, between February 1996 and December 2006, underwent genetic counseling evaluation for HNPCC, and/or whose tumors had microsatellite instability (MSI-H), and/or loss of protein expression on immunohistochemistry (IHC) analyses at M.D. Anderson Cancer Center. Patients were classified into three study groups according to their MMR gene status.

Results: Seventy-one patients had an MMR gene mutation (mutation-positive group), 12 had a variant of unknown significance (VUS group), and 40 had no mutation clinically identified, but did have microsatellite instability and/or abnormal immunohistochemistry protein expression (mutation-negative group). hMSH2 alterations were present in 50 mutation-positive patients and 6 VUS patients; hMLH1 alterations were present in 18 mutation-positive patients and 5 VUS patients; and hMSH6 alterations were present in 3 mutation-positive patients and 1 VUS patient. Males were diagnosed with colorectal cancer at an earlier age than females. Patients in the mutation-positive group had the oldest age at sentinel cancer diagnosis and oldest age at first colorectal cancer diagnosis among the three study populations ($p=0.0054$; $p=0.0003$). Individuals with non-truncating mutations had a younger age at sentinel cancer diagnosis compared to individuals with truncating mutations ($p=0.0488$). An hMSH2 VUS (G683R) occurred exclusively in patients of African American decent and this may represent a population-specific mutation.

Conclusions: The results of this investigation suggest that our study population elucidated both phenotypic similarities and differences compared to what is currently reported in the HNPCC literature. Further studies evaluating large, heterogeneous North American patient populations and mutation-specific genotype-phenotype correlations are warranted.

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