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Clinical Science Podium Presentations

[A1]

Clinical phenotype of individuals with germline mutations in the *PMS2* gene

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Key words: *PMS2*, Lynch syndrome, HNPCC

Background: Individuals with Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer syndrome; HNPCC) have an increased risk of developing multiple types of cancer, especially colon and endometrial. Germline mutations in one of four mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*) cause Lynch syndrome, which accounts for 2-5% of all colon cancers. Fewer Lynch syndrome associated mutations in *PMS2* have been described in comparison to the other MMR genes. This is due at least in part to the difficulty in interpretation of results of *PMS2* mutation analysis given a large family of pseudogenes located on the same chromosome. However, using long range PCR and multiplex ligation probe-dependent amplification (MLPA), germline mutations in *PMS2* have been accurately identified. The identification of these mutations has created a cohort of *PMS2* mutation carriers which allows us to describe the clinical phenotype of *PMS2*-associated Lynch syndrome.

Methods: 28 individuals (26 probands with colon cancer and 2 probands with endometrial cancer) whose immunohistochemistry (IHC) results showed absence of *PMS2* protein and presence of MLH1, MSH2, and MSH6 proteins were tested for germline mutations in the *PMS2* gene using long range PCR, sequencing, and MLPA. Tumors from 23 of these participants showed microsatellite instability (MSI) while MSI results were unavailable for the remaining 5 individuals. Complete clinical data and family history data were available for all participants.

Results: Of the 28 individuals in this series, 20 (71.4%) were found to have deleterious or putatively deleterious germline mutations in the *PMS2* gene. Two of these individuals were found to be compound heterozygotes for germline *PMS2* mutations. Of the 20 individuals with mutations, 13 met Bethesda criteria (65.0%), 2 met Amsterdam I criteria (10.0%), and 5 did not meet any published family history criteria for HNPCC (25.0%). Four of the 20 mutation carriers had a parent with an HNPCC-associated tumor. Samples from at least one parent or other informative relative were available for testing in 5 of the mutation carriers and mutations were identified in one parent/relative in each of these cases. Ages of cancer diagnosis in mutation carriers range from age 14 to 82 with a mean of 48.0 years.

Conclusions: Clinical characteristics of *PMS2* germline heterozygotes have been rarely described to date given the difficulty in identifying these mutations. Based on the current results it is suggested that families that have *PMS2* mutations do not often fit Amsterdam criteria for HNPCC. There are several possibilities for this observation. It is possible that *PMS2* has significantly reduced penetrance. This has been previously suggested in case reports of *PMS2* compound heterozygotes and homozygotes and is supported by evidence in this series of probands in which only 4 of the 20 mutation carriers (20%) had an affected parent with an HNPCC-associated malignancy. Some researchers have suggested that *PMS2* could have a higher de novo mutation rate than the other MMR genes. At least one parental sample was tested for 5 of 20 mutation carriers and a parent was found to have the *PMS2* mutation in each case, providing no evidence of de novo mutation. None of these 5 mutation carriers had an HNPCC-associated malignancy. This too adds circumstantial evidence to the potentially reduced penetrance in *PMS2*. As we gain more data from *PMS2* mutation carriers and further evaluate the penetrance of *PMS2* mutations,

it may become necessary to reconsider the surveillance and screening recommendations for families with *PMS2* mutations.

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[A2]

Current Practices and Opinions of Cancer Genetics Professionals Regarding *MYH* Genetic Testing

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Key words: *MYH*, genetic testing, current practices

Background: There is controversy among cancer genetics professionals regarding when new genetic tests for hereditary cancer syndromes should be offered clinically and what constitutes a clinically relevant genetic test. Genetic testing for *MYH* is commercially available, but the clinical utility of this test and the parameters of its application remain unclear. To help reach a cogent practice consensus we surveyed cancer genetics healthcare professionals from November 2005 to January 2006 about their *MYH* genetic testing practices, the rationale for these practices, and opinions regarding clinical utility.

Methods: Members of the National Society of Genetic Counselor Familial Cancer Counseling Special Interest Group, the Oncology Nursing Society Genetics Special Interest Group, the International Society of Nurses in Genetics, the National Cancer Institute's Cancer Genetics Services Directory, the Collaborative Group of the Americas on Inherited Colorectal Cancer, and Southern California, Colorado, Ohio, and Oregon/Washington Kaiser Permanente Genetics Departments were contacted three times by email with the invitation to participate and link to the online survey. Statistical analyses (not all reported) included ANOVA, Tukey HSD for multiple comparisons, Cronbach's alpha, Pearson Correlation, and Pearson Chi-Square test.

Results: Of 336 responses, 58% were genetic counselors, 25% were nurses, and 10% were physicians. Among the 77% who had seen a patient(s) for polyposis evaluation, 69% had ordered *MYH* testing. The two primary reasons for ordering *MYH* genetic testing were to provide information for family members (96%) and that the patient's screening and risk behavior may be modified by the knowledge (93%). The primary reasons for not ordering *MYH* analysis were unclear healthcare recommendations for monoallelic (72%) and biallelic (64%) *MYH* carriers (72%), and that the patient was not interested (68%). For a patient with 5 colorectal adenomas at age 40 with a negative *APC* analysis and no significant family history, 21% would order *MYH* analysis, 30% would not, and 49% were uncertain. For the same scenario but with 20 or 100 adenomas, 73-78% would order *MYH* testing, 2% would not, and 21-25% were uncertain. For a patient with colorectal cancer at age 40, HNPCC ruled out, and no polyposis or significant family history, 19% would order *MYH*, 35% would not, and 46% were uncertain. When asked if a new mechanism should be developed to provide guidance for when newly available cancer genetic tests should be used clinically, 70% responded in favor.

Conclusions: Our findings indicate that *MYH* ordering practices and rationale are fairly consistent with the published literature but that providers are uncertain of the specific parameters within which to utilize this test. Additionally, the majority of participants expressed interest in test specific guidelines. Given the paucity of such guidelines for *MYH* genetic testing, the CGA-ICC may wish to consider publishing a consortium policy statement.

Funding: This research was funded in part by the National Society of Genetic Counselors Familial Cancer Special Interest Group Grant Award.

[A3]

Evaluating Lynch Syndrome in Very Early Onset Colorectal Cancer Probands without Apparent Polyposis

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Key words: Lynch syndrome, early onset colorectal cancer, diagnostic strategies

Background: Early age of colorectal cancer (CRC) onset is a hallmark of Lynch syndrome and is commonly used as a selection tool when identifying individuals suspicious for germline mismatch repair (MMR) mutations. Previous studies have suggested that probands with CRC diagnosed at less than age 36 have a high likelihood of carrying an MMR mutation regardless of family history. Debate exists as to whether evaluation of Lynch syndrome in these individuals with early onset CRC should begin with germline genetic

testing verses tumor analysis (microsatellite and/or immunohistochemistry analysis for MMR genes). Here we assess probands with early onset CRC for Lynch syndrome and discuss the potential expanded differential diagnoses of MYH-associated polyposis (MAP), attenuated familial adenomatous polyposis (AFAP) and Li-Fraumeni syndrome.

Methods: We evaluated 96 probands with CRC diagnosed prior to age 36 without apparent polyposis using populations from two high-risk cancer clinics and one cancer genetics registry. Probands were categorized based on clinical criteria met (revised Bethesda guidelines I, II, III, IV and V, and Amsterdam criteria I and II) in addition to tumor analysis and MMR germline genetic testing results. Mutation frequencies were calculated for each set of clinical criteria and diagnostic strategies (germline testing verses a staged approach beginning with tumor analysis) were compared.

Results: Out of 89 probands with family history data available, 48 (53.9%) met only revised Bethesda guideline I (CRC diagnosed prior to age 50 years) with or without revised Bethesda guideline III and thus were considered "single case indicators". For 38 of these single cases, evaluation for Lynch syndrome started with tumor analysis while7started with germline genetic testing of the MMR genes (2 probands did not undergo testing and one participant was excluded due to lack of genetic testing after an abnormal tumor analysis). Three of 45 (6.7%) single case indicators were identified to carry a deleterious or suspected deleterious MMR mutation compared with 10 of 20 (50.0 %) in the cases meeting only revised Bethesda guidelines II, IV, and/or V, and 11 of 14 (78.6 %) in the cases meeting Amsterdam criteria. After evaluation for Lynch syndrome revealed no MMR mutations, three families were identified to have additional clinical features suspicious for alternate genetic diagnoses and were documented to have a germline *MYH*, *APC* or *TP53* mutation.

Conclusions: It appears from our data that single cases of very early onset CRC infrequently have identifiable germline MMR mutations. Furthermore our findings suggest that testing strategies in these probands should begin with a staged analysis of MSI and/or IHC followed by germline genetic testing when indicated. Careful attention to evolving or additional clinical features (e.g. polyps and other cancers) is warranted in these cases and may lead to an alternate genetic diagnosis in CRC cases diagnosed prior to age 36. Future studies are needed to better define the frequency of *MYH*, *APC* and *p53* germline mutations in these early onset CRC probands.

[A4]

Population-based analysis of cancers in large Utah families with known mismatch repair gene mutations

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Key words: HNPCC, cancer risk, population-based

Background: Hereditary non-polyposis colorectal cancer (HNPCC) is caused by mutations in DNA mismatch repair (MMR) genes and is hallmarked by incomplete penetrance of early onset colon and uterine cancers. Pancreatic, small intestine, stomach, urinary tract, and ovarian cancers are also associated with this syndrome. Interestingly, the cancers appear to demonstrate geographic variations in penetrance possibly due to differences in genetic pools or environmental factors. Current estimates of cancer risks for HNPCC have been developed mainly from families seeking medical advice. This can lead to biased estimates due to self ascertainment, small family size, lack of communication within families, and the inability to obtain medical documentation spanning several generations. The goal of this study is to examine the prevalence of all cancers in HNPCC families using large Utah pedigrees with confirmed MMR mutations as compared to the Utah population. Probands were ascertained through the Familial Colon Cancer Registry. The Utah Population Database (UPDB) includes 1.6 million genealogy records linked to 136,000 cancer cases from the Utah and Idaho Cancer Registries dating back to 1967, as well as Utah birth and death certificates.

Methods: MMR mutation positive probands representing 13 kindreds linked to UPDB genealogies were identified. The most informative founder of the HNPCC kindreds, going back as far as 5 generations, was selected by evaluating the increased prevelance of colon and uterine cancers diagnosed between 16 and 60 years in the kindred as compared to the Utah population. The minimal p-value combined with pedigree evaluation for linear transmission was used in selection of the founder. Once the founders were identified, the observed versus expected number of cancers in all individuals descending from the founding couple were extracted from UPDB.

Results: The 13 HNPCC families had mutations in MLH1, MSH2, and PMS2; the exact mutations are being verified through medical records. Pedigrees ranged from 2 to 6 generations with an average of

Table 1. (A4)

Cancer	Observed	Expected	Ratio Obs/Exp
Mouth	3	0.861	3.48
Thyroid	2	0.889	2.25
Small intestine	2	0.273	7.33
Colorectal	34	4.271	7.96
Anal	1	0.142	7.05
Bone	1	0.242	4.13
Uterine	10	1.385	7.22
Kidney	4	0.860	4.65
Ureter	1	0.052	19.12
Bladder	5	1.740	2.87
Renal Pelvis	2	0.084	23.84
Other	13	5.888	2.21

123 members (range 5-592). Cancers in 2-fold excess are listed in the adjacent table.

Conclusion: Population-based data from UPDB give an unbiased perspective of cancer risks in large HNPCC families. Colorectal, uterine, small intestine, pancreatic, and urinary tract cancers were found in statistically significant excess. Interestingly, ovarian cancer is not in excess. One striking observation was that 2 of the 3 mouth cancers were of the parotid gland.

[A5]

Transitions to Adulthood in Individuals with FAP

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Familial adenomatous polyposis (FAP) is a paradigm for predictive genetic testing in childhood. This exploratory study aims to identify and describe the psychosocial, reproductive, and medical decision-making issues that arise as children with FAP enter adulthood. Young adults represent an important age group in which to study the impact of genetic knowledge since the responsibility of health care management is undertaken for the first time and important decisions with life-long ramifications are made.

Methods: Validated quantitative questionnaires and semi-structured qualitative interviews were used to explore young adults' (age 19-28) perceived disease burden, FAP-specific knowledge, self-concept, social support, and decision-making regarding education, career, living situations, relationships, reproduction, and medical care. Interviews were coded using a novel theoretical framework that combined models of self--concept and risk-resiliency theory.

Results: A total of ten young adults and eight parents participated in the study. The majority of participants reported positive opinions of themselves; however, there were concerns in three major domains: independence, peer approval, and reproduction. Financial strains, living situations, and poor health contributed to loss of independence. Peer approval concerns were related to perceived undesirability to the opposite sex, negative body image, fear of rejection, and appearing less competent. Reproductive concerns included fearing feeling guilty, not wanting to put a child through what they went through, and not feeling healthy enough to carry a pregnancy. We noted that those with positive outlooks toward FAP tended to be adaptive, goal--oriented, had a strong sense of self, strong social support, and were not influenced by what other people thought of them. 30% of participants did not seem to be receiving appropriate medical care for reasons including financial/insurance strain and lack of medical savvy and self-advocacy. The findings from this descriptive study demonstrate a need for better preparation and support as individuals with FAP transition to adulthood.

[A6]

The Intricacies of Making a HIPAA Compliant Authorization Form for a Research Registry

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Key words: HIPAA authorization, medical record release, and privacy

Background: In short, the Health Insurance Portability and Accountability Act (HIPAA) and its Privacy Rule controls the way a health organization or covered entity safeguards an individual's (living or deceased) protected health information (PHI). HIPAA legislation was passed in 1996 and the compliance date for the Privacy Rule component of HIPAA was April 14, 2003.

Although researchers and clinicians are bound by these regulations, these rules are commonly misun-

derstood, misinterpreted, and consequently misrepresented to patients and research participants. Some health care providers may falsely assume that their current documents, including medical record release forms, are HIPAA compliant, especially when they are Institutional Review Board (IRB) approved. A question about the HIPAA compliance of a medical record release form that was IRB approved prompted the Familial Colon Cancer Registry (FCCR) to re-examine the HIPAA regulations and modify their authorization documents.

Methods: To develop an HIPAA compliant medical record authorization form, the HIPAA documents were reviewed thoroughly and interpreted by the FCCR staff and HIPAA compliance officers at the University of Utah. The HIPAA Privacy Rule was reviewed and the details of the authorization form and informed consent were outlined. After considerable discussion, review, and editing, a release form was approved and is now in use by the Registry and other studies in the High Risk Cancer Clinics at Huntsman Cancer Institute.

Results: From this evaluation, a detailed explanation of the proper way to obtain authorization and informed consent and the components needed to produce a compliant HIPAA medical record release form were compiled. An example HIPAA compliant medical record authorization form has been established. Registry coordinators across the country will be able to use this easy to follow summary to bring their authorization forms into compliance.

Conclusions: To the lay person, the HIPAA Privacy Rule is 33 pages of convoluted language written from a lawyer's perspective. It is designed to protect patients and their identifiable and protected health information. Unfortunately, the language is so difficult to decipher that even seasoned researchers and clinicians often do not fully understand the intricacies of the Privacy Rule. Furthermore, translating the confusing HIPAA language to patients is often a mind-boggling experience. To fully understand HIPAA and the protections it entails to patients, this project involved detailing the HIPAA regulations and each of their components into an easy to follow format. A compliant medical record release form was also produced. It is the hope that this example document can be used by registries across the country. Of note, individual research centers may have slightly varying regulations. Local HIPAA compliance officers can be a beneficial addition to the team when addressing these issues.

Basic Sciences Abstract Podium Presentations

[A7]

Small pool PCR analysis of MSI in familial colorectal cancer type X identifies an attenuated MSI phenotype suggesting hypomorphic mutation in mismatch repair genes as the basis

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Key words: MSI, small pool PCR, familial colorectal cancer type X

Background: Nearly half of families meeting Amsterdam-1 (AC-1) criteria for hereditary nonpolyposis colorectal cancer (HNPCC) do not show microsatellite instability (MSI) by traditional assays, nor have mutations or expression defects in mismatch repair (MMR) genes been identified in them. They have been designated "familial colorectal cancer type X" (FCCX) (JAMA 2005; 293: 1979). We have recently developed a sensitive and quantitative assay for MSI, small pool PCR (SPPCR), which has been shown to be sensitive enough to quantify low but significant levels of MSI in the PBLs of normal individuals (Mech Aging Dev 2005; 126: 1051). There has been little comprehensive analysis of MSI by SPPCR in the various categories of non-polyposis colon cancer.

Purpose: Determine if MSI exists in FCCX tumors and suggest a genetic basis for it.

Materials and methods: Using SPPCR, we analyzed paired blood and tumor DNA from 10 unrelated FCCX patients, alongside age and gender-matched normal control PBL DNA, and compared the data with those from paired blood and tumor DNAs of 7 HNPCC MSI-H patients having different MMR germline mutations and the PBLs of 8 sporadic CRC patients. The *D2S123*, *D5S356*, and *D17S518* loci were amplified using hemi-nested PCR, looking at >112 replicates of ~0.75 genome equivalents per locus per tissue. Mutant fragment (non-progenitor) frequencies (MF) and 95% confidence intervals (CI) were calculated using the statistical programs of SPPCR and DSTATTAB, developed at MDACC.

Results: While the study on the FCCX matched tumor and PBLs is ongoing, of the first 10 patients for which we now have at least 300 fragments analyzed over all 3 loci, the MF range was 0.05-0.18 for the tumor samples (mean 0.086, ±0.029 CI) whereas for the patients' matched PBLs it was 0.01-0.03 (mean 0.016, ±0.011 CI) and for the age-matched normal control PBLs it was 0.015, ±0.004 CI. The PBL data were no different from our 24 historical controls from normal individuals. The MF level of MSI in FCCX tumors was lower than, and below the limits of detection by standard PCR of, that seen in MSI-H tumors (0.236, ±0.133 CI) for these same loci. The MSI level in PBLs of MSI-H HNPCC patients (MF 0.107, ±0.052 CI) was significantly higher than seen in the PBLs of FCCX patients (above). Finally, MFs in the PBLs of the FCCX patients were no different than those seen in the PBLs of the 8 sporadic CRC patients (0.011, ±0.009 CI).

Conclusions: SPPCR reveals significant levels of MSI in FCCX tumors, yet they are lower than seen in HNPCC-MSI-H tumors. Also, MSI-H individuals show significant MSI in their PBLs while the FCCX patients do not. These results suggest that FCCX might be due to MMR missense or hypomorphic mutations having less severe consequences than those seen in the MSI-H patients. Alternatively, mutation in a gene in the MMR pathway less proximal to the repair event might evoke this attenuated MSI phenotype. Other possibilities will be discussed. Screening of FCCX patients for such mutations might now be valuable for identifying genetic markers increasing cancer risk.

[A8]

Gonadal Mosaicism in a Family with Familial Adenomatous Polyposis

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Key words: mosaicism, familial adenomatous polyposis (FAP), haplotyping

Background: Individuals who are mosaic for genetic mutations are rare, although they have been reported in a number of genetic conditions including Duchenne

muscular dystrophy and Turner syndrome. Mosaicism is the result of a mutation that occurs early in embryonic development. The mutated cell goes on to produce tissues in the body that have the mutation; the rest of the stem cells do not have the mutation and develop into unaffected cell lines, causing an individual to be a mixture of cell types. Gonadal mosaicism causes an individual's egg or sperm to be a mixture of cells with and without the mutation. This case demonstrates the unique situation of gonadal mosaicism for a solitary de novo mutation in *APC*.

Methods: In our clinic, we identified a nuclear family with two adult children affected with familial adenomatous polyposis (FAP), confirmed by DNA sequencing of the *APC* gene. Each affected child was heterozygous for the deleterious mutation c.G4729T (E1577X). Each parent and three remaining siblings were determined to be unaffected, both by polyp-free colonoscopy and by normal sequencing results for the G4729T mutation. Paternity and maternity of all the children were reported by the parents. To determine if parental mosaicism caused the two affected siblings, *APC* haplotyping was conducted. Allele-specific oligonucleotide (ASO) PCR is being used to determine the parent of origin. If necessary, paternity testing will be conducted.

Results: Haplotyping of DNA, obtained from peripheral blood samples from the parents and the children, of 4 polynucleotide markers flanking the *APC* locus, demonstrated that all five children had inherited the same *APC* allele from their father (designated "A") (see Figure 1). Both affected children and one unaffected child share one of the two maternal alleles (designated "C"). ASO-PCR is being used to individually amplify the maternal and paternal alleles from the affected and unaffected children; DNA sequence analysis will determine whether the G4729T mutation arose on the paternal or maternal allele.

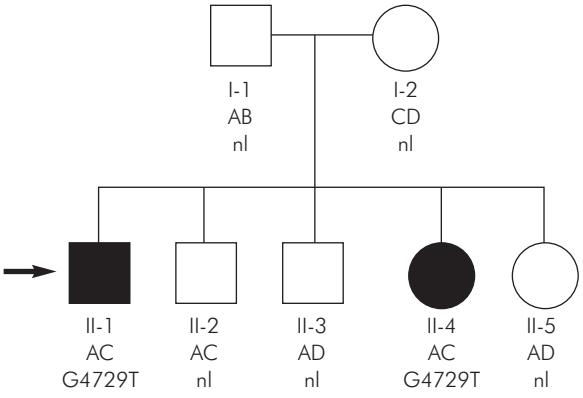

Figure 1. Pedigree showing genotypic and phenotypic information (A8)

Conclusion: The actual incidence of FAP mosaicism in the general population is unknown. Even though an individual may appear to represent a de novo mutation, where parents and siblings would not be at risk for the condition, clinicians need to consider the possibility of gonadal mosaicism. For this reason, it is imperative that *both* the parents and siblings of an isolated affected individual be tested for the specific mutation.

[A9]

Common Familial Colorectal Cancer Linked to Chromosome 7q32.33: A Sibpair Study

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Collaborative Group of Americas on Inherited Colorectal Cancer, Nashville, TN 2006

Key words: sibpair; linkage; colorectal cancer

Background: Colon cancer is one of the most familial of all cancers with present investigations suggesting that 20-35% of cases arise on the basis of inherited factors. Genetically defined inherited conditions, however, account for less than 5% of colorectal cancers. We hypothesize that the remaining inherited factors are moderately penetrant genes that are common in the population. We use an affected sibling pair approach to identify genetic regions that are coinherited by siblings with colorectal cancer at an increased rate from what would be statistically expected by chance.

Methods: Individuals from families with at least two siblings diagnosed with colorectal adenocarcinoma or carcinoma in situ were enrolled, medical records obtained, and DNA collected. Known syndromes were excluded by medical record review and HNPCC by MSI analysis followed by sequencing when tumor blocks were available. A genome-wide scan on 152 samples representing 70 kindreds was completed using deCODE's 1000 STR marker set at an average 4 cM density. Fine mapping on a total of 186 DNAs (163 affected and 23 unaffected) from 83 kindreds was done using deCODE markers at a 1 cM density. Linkage analysis was accomplished using MERLIN analysis package. Analysis was run using allele frequencies of two populations 1) the study participants and 2) the Caucasian HAPMAP.

Results: MSI analysis was completed on 87 colon cancer cases (25 MSI-H, 56 MSS, 6 MSI-L). 11 kindreds

Table 1. Pathology of siblings sharing the 7q31 allele (170 sib-relationships) (A9)

	All cases	0-shared alleles	1-shared allele	2-shared allele
Number of cases	163	26	106	38
Average age diagnosis	58.3 years	59.2	59.1	57.9
Right-sided cancers	42%	27%	44%	26%
Cancer Grade (G1:G2:G3)	11%:70%:19%	13%:75%:13%	12%:76%:12%	12%:72%:16%
Lymph node involvement (N1+N2)	30%	32%	33%	28%

have been sequenced for MLH1 and MSH2; 6 are negative for mutations; 5 have a disease-causing mutation and were excluded from linkage analysis. The racial make-up of the individuals in linkage was 87% white, 4% black, 1% Native American, and 8% other. No significant linkage was found near genes causing known syndromes or chromosome 9q22, a region previously reported by other groups. Linkage analysis revealed three significant genetic regions: Chr. 4 (D4S3046) at 159 cM LOD 1.38 (p=0.003); Chr. 7 (D7S669) at 91 cM LOD 2.52 (p=0.0003); and Chr. 7 (D7S2418) at 122 cM LOD 1.74 (p=0.002). Fine mapping confirmed the chr. 7q31 region, with the peak at 130 cM with an LOD of 3.00 (p=0.0001).

Conclusions: Investigation of sibling pairs with colorectal cancer identified statistically significant linkage to chromosome 7q31. No known familial cancer genes reside here; thus the identified region may contain a novel susceptibility gene responsible for common colorectal cancer. Affected siblings with increased sharing of this locus have a slightly earlier age of onset (1.2 years) and a slightly lower rate of metastasis to the lymph nodes. Other pathologic features did not show a trend with increased sharing.

[A10]

The prevalence of fundic gland polyps and dysplasia in familial adenomatous polyposis

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Key words: fundic gland polyps, dysplasia, familial adenomatous polyposis

Purpose: Upper GI cancer is the leading cause of mortality in FAP patients after colectomy. Fundic gland polyps (FGPs) have been considered non-neoplastic; however, cases of gastric carcinoma have been reported arising from them. Our study aims to 1) determine the prevalence of FGPs and FGP dysplasia in FAP and 2) identify whether any endoscopic or demographic features are associated with the presence of FGP dysplasia.

Methods: This prospective, IRB-approved study enrolled consecutive FAP patients who presented to the Cleveland Clinic for upper endoscopic surveillance. Demographic information and medical history were collected. On EGD the presence or absence of FGPs was noted. FGP size (1-5 mm, 6-10 mm, >10 mm), FGP number (1-20, 21-30, >30), duodenal polyposis stage, and H pylori status were determined. Systematic biopsies of FGPs were obtained. Histologic analysis was performed by one GI pathologist to assess for the presence of FGP dysplasia, which was classified as: negative for dysplasia; indefinite for dysplasia; low-grade dysplasia; or high-grade dysplasia based upon a priori definitions and standard clinical practice. Univariable analyses were performed to determine associations between dysplasia and: family history of gastric cancer; tobacco use; use of NSAIDs or acid suppression therapy; presence of Helicobacter pylori (HP); number and size of FGPs; and stage of duodenal polyposis. Multivariable logistic regression analysis was used to study the associations of demographic and/or endoscopic factors with presence of dysplasia in FGPs.

Results: 40 subjects were enrolled in the study with a mean age at time of exam of 42.8 (±12.5) years and 55% were males. 33/40 subjects (82.5%) had FGPs. Subjects with FGPs were less likely to have H. pylori infection (3% vs. 42.9%; $p=0.013$).

18/33 subjects (54.5%) had LGD or were indefinite for dysplasia (15 LGD/3 indefinite).

Univariable analyses reveal no significant association between FGP dysplasia and: family history of gastric cancer; tobacco use; use of NSAIDs; acid suppressive therapy; presence of HP; number and size of FGPs; or stage of duodenal polyposis. In the

multivariable analysis subjects who did not use acid suppressive therapy are 6.7 (95% CI: 1.05, 43.01) times more likely to have dysplasia than those who did. Additionally, subjects with increasing numbers of FGPs (OR 3.07; 95% CI 0.98, 9.62) and higher

Spigelman duodenal polyposis stage (OR 1.91; 95% CI 0.87, 4.19) are more likely to have dysplasia.

Conclusion: FGPs are seen in the majority of patients with FAP. There is a negative association between FGPs and H pylori. Dysplasia is seen in more than 50% of subjects with FGPs. Dysplasia is more likely in subjects not taking acid suppressive therapy; with increasing numbers of FGPs; and with higher Spigelman duodenal polyposis stage. Continued prospective evaluation of this cohort of patients will allow us to garner more knowledge about the natural history of FGP dysplasia.

Funding: American College of Gastroenterology research grant.

[A11]

Microsatellite unstable (MSI) human colorectal cancer (CRC) is characterized by increased epidermal growth factor receptor (EGFR) gene expression which is strongly associated with mutations in the poly A tract of the 3′ untranslated region (UTR) of the EGFR gene

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Key words: colorectal cancer, microsatellite instability, EGFR

Introduction: EGFR-targeted molecular therapies have shown significant but modest effectiveness against human CRC. We recently reported statistically significant ($p=0.001$) increased EGFR expression in MSI vs. microsatellite stable (MSS) human CRC cell lines.* Our present study tests the hypothesis that increased EGFR expression in MSI CRC results from 1. Increased EGFR gene copy number and/or 2. an EGFR gene coding sequence alteration and/or 3. a 3′ UTR transcription regulatory sequence alteration.

Methods: FISH (fluorescent in-situ hybridization) analysis was employed to measure EGFR gene copy number in 11 MSI and 17 MSS cell lines. Automated cDNA sequence analysis was used to search for EGFR gene sequence variations from wild type in 8 EGFR over-expressing MSI cell lines.* Targeted sequence analysis of the Poly A 3′ UTR sequence was performed on all 28 lines.

Results: FISH analysis of the 28 human CRC cell lines including 8 EGFR over-expressing MSI lines found no evidence of EGFR gene amplification. Similarly, sequence analysis of EGFR cDNA in the 8 EGFR over-expressing MSI cell lines demonstrated no sequence alterations from wild type. However, 7 of 8 EGFR over-expressing MSI lines and 0/17 MSS lines demonstrated a del A sequence alteration in the poly A(13) tract of the 3′UTR of the EGFR gene.

Conclusions: We found no evidence to support the hypotheses that gene amplification or cDNA sequence variations were associated with the increased EGFR expression we reported.* However, 88% (7/8) of MSI CRC cell lines characterized by EGFR over-expression demonstrated the del A sequence alteration in the 3′ UTR of the EGFR gene. These results support the hypothesis that 3′ EGFR poly A sequence alterations contribute to EGFR over-expression in MSI CRC. We conclude that these results offer significant insights into the mechanism of increased expression of EGFR in MSI CRC.

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[A12]

Why do colorectal cancer patients have inaccurate perceptions of the risk to their first degree relatives: differences between African Americans and Caucasians

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Key words: colorectal cancer, risk perception, racial/ethnic differences

Background: Results from previous studies revealed that less than 50% of patients diagnosed with colorectal

cancer (CRC) knew that their first degree relatives (FDRs) were at an increased risk for CRC, and the majority could not identify the appropriate CRC screening guidelines for their FDRs. Furthermore, Caucasian patients were significantly more likely to have an accurate FDR risk perception compared to African Americans. This pilot study aims to confirm previous results and to explore factors which influence CRC patient perception of cancer risks to their FDRs and how these factors differ between Caucasian and African American patients.

Methods: Using the survey instrument from previous studies, literature review, and validated study measures, the investigators developed a standardized telephone survey which was administered to 27 CRC patients from the University of Chicago Cancer Registry. The survey focused on six areas: (1) demographic characteristics, (2) knowledge of FDR cancer risk, screening guidelines and information source, (3) beliefs and knowledge about the causes of illness and cancer risk factors, (4) CRC diagnosis experience and patient-physician relationship, (5) level of mistrust in health care and medical professionals, and (6) level of participation in religious and/or spiritual activities and experiences.

Results: Eight African American and 19 Caucasian patients participated in this study (~45% response rate). Approximately half of patients (14 out of 27) understood that their FDRs were at an increased risk for CRC compared to the general population. Although not statistically significant, Caucasians were more likely than African Americans to have an accurate FDR risk perception (63.16% vs. 25%; p=.075). Regardless of race, individuals who had an accurate FDR risk perception tended to: be younger (p=.003), be younger at CRC diagnosis (p=.003), have completed a higher level of education (p=.011), have an annual income of 50,000 or more (p<.001), identify genetics as a CRC risk factor, have more trust in healthcare and medical professionals (p=.022), and have a lower level of religiosity/spirituality (p=.145). Compared to Caucasians, African Americans were more likely to: be older, be older at CRC diagnosis, have an annual income of 50,000 or less, have less knowledge of CRC risk factors, have less level of trust in healthcare and medical professionals, and have a higher level of religiosity/spirituality.

Conclusions: A significant number of CRC patients do not understand that their FDRs are at increased risk for CRC, especially African American patients. Future studies are needed to measure factors that influence FDR risk perception and how these factors vary among different racial groups. This study suggests that health care professionals should be aware that CRC patients may not understand their familial risks and that both racial and socioeconomic background should be considered when providing patient education about familial CRC risk.

[A13]

Is There Something About a New Mutation That Causes Severe Disease in FAP? A Comparative Study of Probands Stratified by Family History

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Purpose: Approximately 25% of FAP patients have no family history of the disease. Little is known about disease expression and genotype in these patients as previous studies have been flawed by the inclusion of multiple screened relatives in the control group. To avoid this bias only probands were included in our study. We have investigated the influence of lack of antecedent family history on the clinical and genetic characteristics of FAP.

Methods: A retrospective review of 176 consecutive probands with FAP using the Polyposis Registry at the Cleveland Clinic was carried out. Adequate pedigree data for at least three generations were available for 50 new mutation and 73 control patients.

Results: Mean age at diagnosis did not differ between patients and controls (31 and 33 years, p=0.48). Patients without family history presented with twice the incidence of colorectal cancer (32% vs. 16%, p=0.04) and two and a half times the incidence of desmoid tumors (40% vs. 17%, p=0.008). CRC developed at a significantly younger age in new mutation patients (33 vs. 45, p=0.027). No significant difference between the two groups was found for the number of colorectal polyps (<100, 100-1000, >1000), presence of gastro-duodenal polyps and other extra colonic manifestations and malignancies. Overall 4 (8%) patients without family history and 7 (9.6%) patients in the family group had died (p=1.0) at a mean age of 44 and 53 years (p=0.19) and 6 and 7.5 years of follow-up respectively. 24 patients were tested for APC germline mutation. Detection rate was similar between the two groups and no specific APC variation was over-represented in either group.

Conclusions: Significantly higher rates of CRC, desmoid tumor and malignant transformation at younger age indicate a tendency toward a more severe form of polyposis in FAP patients with no family history. These findings are unlikely to be due to a delay in diagnosis

and no phenotype-genotype correlation was observed. Despite the increased severity, new mutation apparently does not lead to an increase in mortality in FAP.

[A14]

How aware are colorectal surgeons of hereditary colorectal cancer, and how much does it impact their practices?

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Background: Hereditary colorectal cancer is said to be rare and its rarity is a reason commonly given for advising physicians to refer such patients to a genetics center or a registry. We designed this study to discover just how often colorectal surgeons see patients with hereditary colorectal cancer, and to measure their level of interest in this topic.

Methods: A questionnaire was designed that asked how interested the surgeon was, the number of patients and families seen with the different syndromes each year, and the number of colectomies performed for these syndromes each year. Results were stratified according to the presence of a special interest in hereditary colorectal cancer (HCC). The questionnaire was sent to the 41 training programs in colorectal surgery and was distributed at a recent meeting of the American Society of Colorectal Surgeons.

Table 1. (A14)

	Special Interest	No Special Interest
N	45	36
Heard of LCPG	28 (62%)	16 (44%)
Heard of ICG-HNPCC	34 (76%)	16 (44%)
Heard of CGA	16 (36%)	12 (33%)
Heard of InSiGHT	8 (18%)	8 (22%)
Member of any Group	3 (7%)	0
FAP Pts/Families per surgeon per year	4.0/2.0	1.2/0.8
HNPCC Pts/Families per surgeon per year	4.1/3.3	2.1/1.7
Colectomy/proctocolectomy per surgeon per year	4.1	1.6
Orders Genetic Testing	82%	56%
Orders Pre-test counseling	71%	56%

Results: 81 questionnaires were returned; 25 from surgeons directly involved in an HCC center or registry and 56 from surgeons who were not. Results in the table are stratified according to whether the surgeon had a special interest in HCC or not.

Conclusions: Colorectal surgeons can expect to see 3 to 8 patients with HCC per year. Many have a special interest in these syndromes and play an active role in their care. Others without this interest should refer their patients to specialized centers for help with counseling, testing and management.

[A15]

An atypical hereditary non-polyposis colorectal cancer syndrome (HNPCC) family presentation

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Key words: MLH1, carcinosarcoma, gynecologic

Background: It is well established that patients with HNPCC are at increased risk for colon cancer and it is often thought of as a colorectal cancer dominated syndrome. HNPCC has also been associated with an increased risk for extra-colonic cancers and multiple primary cancers; therefore identifying individuals with HNPCC can have a major impact on future screening and medical management options.

Methods: We describe a unique HNPCC family and the role that genetic counseling and testing played in the management of the patient.

Results: The patient is a 42-year-old white female who presented to her gynecologist with vaginal spotting and cramping. A pelvic exam revealed a large mass and the patient was referred to gynecologic oncology for surgical evaluation. The patient underwent total abdominal hysterectomy, bilateral salping-oophorectomy and lymph node dissection. The pathology revealed a Stage IIA endometrial carcinosarcoma (EC) with zero of seven lymph nodes positive and the patient was treated with chemotherapy. During her treatment, the patient asked to meet with a genetic counselor to discuss her family history. The patient's family history was consistent with the Amsterdam criteria as her father was diagnosed with colon cancer at 35 and her aunt was diagnosed with both colon and uterine cancer. Unfortunately, the family history was not recognized prior to the patient's diagnosis. Full sequencing of MLH1 and MSH2 revealed a deleterious MLH1 mutation (IVS6-2A>G) and the implications of the testing for the patient and her at-risk

family members was discussed. Due to the patient's mutation status and high risk for colon cancer, colonoscopy was recommended and performed and revealed a 6cm cecal mass. The patient underwent a total abdominal colectomy with an ileorectal anastamosis and pathology was consistent with a primary colon cancer, which was diagnosed less than one year after her EC. To date, her five siblings and four children remain asymptomatic and have not had genetic testing despite the urging of our patient.

Discussion: Our patient appears to be one of the first reports of a carcinosarcoma in an MLH1 family.

The patient's father developed laryngeal cancer (a second primary) at the age of 47, which is not thought to be associated with HNPCC and brings into question the spectrum of HNPCC cancers. All of the mutation-positive individuals in this family have developed multiple primary cancers (including two women with both colon and uterine cancer) which make us question whether certain mutations carry higher risks for multiple primary cancers than others. This information would have great impact on the screening and management of individuals in these families that may carry these mutations.

[A16]

Two brothers with Muir-Torre syndrome and biallelic MYH mutations

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Key words: MYH associated polyposis, Sebaceous gland tumors, Muir-Torre syndrome

Background: Muir-Torre syndrome (MTS) is clinically defined by the presence of sebaceous tumors and visceral malignancies, usually colorectal cancer (CRC). It was suspected to be a variant of hereditary non--polyposis colorectal cancer (HNPCC) and the identification of mutations in the HNPCC-associated mismatch repair (MMR) genes in some patients with MTS seemed to confirm this suspicion. As MMR mutations are not identified in all Muir-Torre patients, genetic heterogeneity cannot be excluded. Recently, a single patient with sebaceous adenomas and MYH-associated polyposis (MAP) was reported, raising the possibility that a subset of these patients may also have MTS. We recently evaluated two brothers with a history of colonic polyposis and CRC who both received extensive treatment by a dermatologist for facial lesions.

Methods: Both brothers underwent a standard clinical assessment in the cancer genetics clinic as well as further dermatological evaluation including biopsies of the facial lesions. Due to the history of colonic polyposis and colorectal cancer, *Colaris* AP testing through Myriad was initiated to screen for mutations in both *APC* and *MYH*.

Results: The older sibling had a hemicolectomy for invasive adenocarcinoma of the cecum and multiple right-sided tubular adenomas. One year later he was found to have hundreds of minute adenomatous polyps in the rectum and has since required an ileal pouch anal anastomosis. His dermatological assessment confirmed the presence of numerous facial papules that on pathological examination were confirmed to be mainly sebaceous hyperplasia or sebaceous hyperplasia with hamartomatous features. One lesion had definitive features of a sebaceous adenoma. His brother had a hemicolectomy for invasive adenocarcinoma of the ascending colon, multiple right--sided tubulovillous adenomas and synchronous adenocarcinoma of the small intestine. Dermatological exam revealed ~50 umblicated facial papules in keeping with sebaceous hyperplasia. *APC* mutation analysis revealed no known pathogenic mutations; however a variant of unknown significant, E1317Q, was found. Both brothers are homozygous for the *MYH* Y165C mutation, which confirmed a diagnosis of MYH-associated polyposis.

Conclusions: We report on two siblings with colon cancer, multiple colonic adenomatous polyps as well as sebaceous gland tumors including a sebaceous adenoma. While *MYH* involvement has focused on individuals with clinical phenotypes suggestive of attenuated familial adenomatous polyposis, the full clinical spectrum of MAP remains to be defined. The finding of sebaceous adenoma/hyperplasia in our two patients provides further evidence of genetic heterogeneity for Muir-Torre syndrome. Sebaceous gland tumors may be a useful cutaneous marker to help identify individuals with either MAP or HNPCC.

[A17]

Cowden Syndrome with PTEN Mutation Presenting as Ganglioneuromatous Polyposis of the Colon with Colonic Adenocarcinoma

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Key words: Cowden, colonic adenocarcinoma, ganglioneuromatosis

Background: Cowden syndrome, first described in 1963, is an autosomal dominant condition characterized by mucocutaneous hamartomas and increased risk of some visceral malignancies, most notably of the thyroid and breast. It is caused by mutations in the PTEN gene.

Methods/Results: We report a case of Cowden Syndrome with characteristic dermatologic and unusual colonic manifestations. The male patient had a colectomy for diffuse colonic polyposis at age 39. Pathology of the colon showed polypoid ganglioneuromatosis, and an incidental invasive mucinous adenocarcinoma was found in one of the polyps, which was associated with adenomatous dysplasia. Although the adenocarcinoma was confined to the head of the polyp, metastatic tumor was found in 3/17 colonic lymph nodes (AJCC T1N1). Several mesenteric lipomas were also noted. He received adjuvant chemotherapy and six years later has no evidence of recurrent cancer. At age 45, he was referred to the dermatology clinic for evaluation of multiple skin lesions including: numerous small papules that were nearly confluent on the nose and nasolabial folds, several keratoses on the dorsal aspect of both hands, a distinctive cobblestone appearance on the tongue and oral mucosa, and a 1 cm dome-shaped nodule on the abdomen. Biopsy of one of the facial lesions revealed a trichilemmoma and the abdominal lesion was a storiform collagenoma. The dermatologic findings meet clinical criteria for the diagnosis of Cowden Syndrome. Analysis of the PTEN gene in germline DNA showed a truncating mutation.

Conclusions: Colonic manifestations are a hallmark of several hamartomatous syndromes related to Cowden Syndrome, but it is rare to find colonic polyposis, intestinal ganglioneuromatosis, and colonic adenocarcinoma in Cowden Syndrome with PTEN mutation. Their presence should not exclude this diagnosis.

[A18]

The Phenotype of MYH-Associated Polyposis? Experience with 6 families

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Background: The full phenotype of MYH-associated polyposis (MAP) is still not known as the syndrome is relatively new and the number of affected families relatively sparse. The syndrome often seems to mimic attenuated FAP except that the pattern of inheritance is recessive rather than dominant. We report a preliminary study of the full tumor spectrum seen in 6 MAP families.

Methods: The families of six probands with MAP were characterized by extensive pedigree building. Where available, pathology reports were requested to document the reliability of the family history. All probands had biallelic mutations of *MYH*.

Results: Excluding relatives under the age of eighteen and the spouses of at-risk relatives, there were 136 at--risk relatives in the 6 families. There were 41 affected (30%), some with multiple tumors. The tumor spectrum is shown in the table.

Discussion: The colorectal phenotype of these MAP families is as expected. 17.6% of relatives had adenomas although less than half of the relatives had screening colonoscopy. Colon cancers were found in 16 individuals, usually with an older age of onset. The spectrum of extracolonic cancers seen in these 6 families is unusual in the setting of MAP. There were no cases of gastric or duodenal cancer, but renal cancer occurred in three different families. There were four cases of prostate cancer in one family and two with pancreatic cancer in another.

Conclusion: The phenotype of MAP is evolving. Detailed studies on phenotype in much large numbers of families are needed. Renal cancer was present in half of these families and must be investigated as a possible component of phenotype.

Table 1. (A18)

	Adenomas	Colon	Prostate	Renal	Uterine	Pancreas	Breast	Bone	Brain	Lung	Leukemia
Pts	24	16	5	3	2	2	1	1	1	1	1
Fams	6	5	2	3	1	1	1	1	1	1	1

[A19]

Hispanics' Knowledge, Attitudes and Screening Behaviors Regarding Familial Colorectal Cancer

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Key words: colorectal screening behavior, Hispanics, familial colorectal cancer

Purpose: Relatives of colorectal cancer (CRC) patients are at increased risk of developing CRC themselves, compared with the general population. Screening recommendations for persons at risk for familial CRC advise earlier initiation of screening, compared with persons at average risk, generally by colonoscopy. Studies of CRC screening behavior among persons at familial risk have found that screening adherence is suboptimal, and most studies have included study populations that are primarily White. Although Hispanics comprise the largest U.S. ethnic minority, there are scant data regarding Hispanics' knowledge, attitudes, and beliefs regarding CRC and CRC screening. Furthermore, little is known about how family communication regarding CRC risk influences adoption of and adherence to CRC screening recommendations among increased-risk persons. This study examined CRC knowledge, CRC screening behavior, and communication with family and physicians regarding CRC in a sample of Hispanic CRC probands and their FDRs.

Methods: We recruited persons who self-identified as Hispanic and who were diagnosed with CRC at age ≤60 years, as well as their FDRs age 40 years or older. Data were collected through in-depth, semi-structured telephone-administered interviews.

Results: We enrolled n=31 participants (20 CRC probands, 11 FDRs). Most participants perceived few barriers to discussing CRC or CRC screening with members of their immediate family, and CRC probands often initiated these discussions. Most FDRs had undergone at least one colonoscopy, many in response to their relative's diagnosis. Despite having personal experiences with CRC, probands demonstrated knowledge deficits regarding screening and prevention guidelines for their FDRs. FDRs also lacked knowledge regarding important CRC risk factors (including polyps) and screening guidelines for individuals with a family history of CRC. FDRs perceived few barriers to discussing family history of CRC or CRC screening with their physicians. However, FDRs generally did not initiate discussions with providers about familial CRC risk and indicated that they would rely primarily on a physician's prompting to undergo screening.

Conclusions: A CRC diagnosis in a close relative may motivate discussion and initiation of screening in Hispanic families. These results are encouraging; however, gaps in knowledge about CRC risk and screening needs, as well as relatively passive attitudes toward initiating screening discussions with providers, may interfere with repeat adherence, and could be addressed through counseling and education. Findings from this study can inform future research and clinical practice regarding CRC screening behavior in this understudied population.

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[A20]

Can the Development of Desmoids be Predicted? The Use of Knowledge Discovery Techniques in Familial Adenomatous Polyposis

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Purpose: Clinically significant desmoid tumors occur in 12 to 15% of patients with FAP. When large or fast growing they cause severe morbidity and even death. Strategies to minimise their impact include delaying prophylactic colectomy, pre-operative treatment with anti--desmoid drugs, and choice of some operations (e.g. laparoscopic IRA) while avoiding others (e.g. laparoscopic pouch). In some patients desmoid risk can be determined by genotype or family history, but the most severe desmoids often come as a surprise. We performed this study to see if knowledge discovery techniques could help predict patients who will develop desmoids.

Methods: Knowledge discovery techniques, clustering and classification were applied to an inherited colorectal cancer database of 557 FAP families with 8,889 family members. We analyzed pedigree relationships, data related to clinical presentation of FAP, test results, and follow-up. The data set was converted to a high dimensional output appropriate for the data mining analysis with 143 attributes for each family member.

Results: Clustering analysis grouped family members into two distinct clusters with a high and a low probability of desmoids. Female patients who have a family history

Table 1. (A20)

Rule	% diagnosed with desmoid
3-5 ECM and surgery: ECE and Prophylactic Colectomy	100
3-5 ECM and surgery: ECE and Dental Abnormalities	100
3-5 ECM and surgery: ECE and Fundic gland polyps	100
Female and surgery: ECE and duodenal adenomas	90
Female and 3-5 ECM and duodenal adenomas	88
ECE and fundic gland polyps and epidermoid cyst	86
3-5 ECM and surgery: ECE and congenital hypertrophy of the retinal pigment epithelium	86

ECE – extra-colonic excision; ECM – extra-colonic manifestation.

of desmoids and have been diagnosed with osteoma, gastric polyps, and epidermoid cyst fall into the cluster with a high probability of desmoid disease. (43 percent in this cluster have desmoids versus 15 percent in a low risk cluster (male patient with no family history of desmoids, no gastric polyps, epidermoid cyst, or osteoma). We also built a rule-based classifier that searched for patterns in the database and represents these in a form of association rules. Most rules incorporate the extracolonic manifestation of Gardner's syndrome. For example, one of the discovered patterns is that 88 percent of female patients with at least three extra-colonic manifestations and duodenal polyps have been diagnosed with a desmoid (Table 1).

Conclusion: The knowledge discovery process has identified a series of FAP phenotypes that are associated with a high risk of desmoid disease. Work-up of FAP patients must include a thorough examination for extra-colonic manifestations. Patients with these phenotypes should be counselled and their care managed appropriately.

[A21]

Taking a Family History can be Fun! Touch-Screen Assessment using the Family History Score

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Background: A family history of colorectal cancer can be complex because of the multiple possible permutations and combinations. A Family History Score has been developed and validated to simplify familial risk assessment. We have applied a program allowing use of touch screen computer technology to the calculation of familial risk of colorectal cancer using this scoring system. In this study we report the results of a test of the program at a women's health fair.

Methods: The Family History Scoring System has been published recently and its use in HNPCC families has been described (Church JM. A scoring system for the strength of a family history of colorectal cancer. Dis Colon Rectum 2005; 48: 889-96. Church JM, McGannon E, Patrick D. Validating the family history score in Amsterdam positive families enhances its use in the general population. Dis Colon Rectum 2005; 48: 659-60.). Patients with scores <8 are considered at low familial risk of cancers or advanced adenomas while scores >7 signify high risk. Scores >11 are suggestive of a hereditary colorectal cancer syndrome.

A program was designed in which family history was entered by answering a series of questions including the relationships and number of affected first, second, and combination first and second-degree relatives to the screener, and age at diagnosis. The program calculated the Family History Score. Each set of scores had associated screening recommendations which were reviewed with the participants. A table was set up at a women's health fair offering a colorectal cancer risk

Table 1. (A21)

Score	Risk level	N
0	Average population	203 (75%)
1-7	Low increased	63 (23%)
8-10	High increased	1 (0.4%)
>10	Likely HNPCC	3 (1%)

assessment. Interested participants were asked questions about their family history of colon cancer and age when affected using the touch-sensitive computer screen. No identifying information was collected. Recommendations for surveillance were given depending on the risk level assigned.

This study was approved by the Institutional Review Board at the Cleveland Clinic.

Results: 270 women participated. The scores are shown in the table. Women found the questionnaire easy to complete and compliance was high.

The three patients with likely HNPCC had scores of 12, 18 and 21. These families need workup by a genetic registry.

Conclusion: the Family History Score is a simple way of measuring familial risk of colorectal cancer and is suitable for use with a computer and a touch-screen. This should improve understanding of familial risk of colorectal cancer and compliance with screening recommendations.

[A22]

Optimizing PCR-based assay for MSH2Δ1-6 American founder mutation

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Collaborative Group of Americas on Inherited Colorectal Cancer, Nashville, TN 2006

Key words: HNPCC, MSH2, PCR

Background: An American founder genomic deletion of exons 1-6 of the MSH2 gene in HNPCC families has been previously characterized (Wanger et al., 2003). As described by Wagner et al., the founder became a Mormon in the 1800s and traveled from Alabama to Utah. Thus, its prevalence is of particular interest in the Utah population. The deletion is thought to arise from a recombination event between two Alu elements resulting in removal of 20 kb genomic DNA including exons 1-6. A deletion-specific PCR-product of 1.7 kb allowed for rapid identification of individuals harboring this mutation. DNA from individuals lacking the mutation yielded no product. Multiple labs including ours found that this PCR reaction was not very robust; we encountered variable results and false negatives. Consequently, we have modified the PCR reaction by changing the Taq DNA polymerase system, one primer, and nesting a second primer that will yield an additional 3 kb band from the normal MSH2 allele.

Methods: Using a known mutation carrier as a positive control, three Taq DNA polymerase systems were examined: recombinant Taq (Fermentas); FastStart Taq (Roche) and PrimeStar HS (Takara). PCR reactions included 0.2 μM primers, 200 μM dNTPs, 50 ng genomic DNA, and Taq, buffers and $MgCl_2$ as recommended by the manufacturer. Thermocycler conditions included a hot-start, then 30 cycles of 94°C 1 min, 57°C 15 sec, and 72°C 2 min.

The modified PCR reaction was run as above with the PrimeStar HS DNA polymerase (0.625u/25ul) and 1xPrimeStar buffer (1mM $MgCl_2$) with the following primers:

R3: 5′ GCTGAATTAGGTTTTGGAAC 3′ (5′ primer upstream of exon 1; Wagner et al., 2003).

P2: 5′ CATAACCCTGCCTAACACAT 3′ (3′ primer specific for wild-type product in intron 1)

F4: 5′ TGCAATTCTGAGAGTCAACA 3′ (3′ primer for deletion product in intron 6)

Results: The PrimeStar HS DNA polymerase clearly gave the best result. 17 individuals from a kindred with the MSH2Δ1-6 mutation were tested using the PCR method described. Mutation carriers yielded two bands of 1557 bp (mutant allele) and 3141 bp (wild-type allele), noncarriers yielded the single band of 3141 bp, and failures had no product. In addition, we surveyed 30 probands from Utah colon cancer sibships and no MSH2Δ1-6 mutation carriers were identified.

Conclusions: We have developed a robust PCR-based assay for screening of the MSH2Δ1-6 American founder mutation. This assay eliminates false negatives through a nested PCR of the wild-type product.

Using this assay, it will be informative to ascertain the actual prevalence of this mutation in a large set of Amsterdam-positive individuals or individuals fulfilling Bethesda Criteria from America.

[A23]

Probands and non-probands differ by age of onset to colorectal cancer in Lynch syndrome

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Key words: Lynch syndrome, screening, age of onset

Background: Lynch syndrome individuals are at an increased lifetime risk of developing colorectal cancer

(CRC) and certain other types of cancer because of an inherited germline mutation in one of a set of genes responsible for DNA mismatch repair (MMR). The median age of cancer onset is ~45 years. However, since most Lynch syndrome families come to light through hospital--identified probands, this estimate may be biased towards a younger age of onset. Clinically, the suspicion of Lynch syndrome arises when individuals present with CRC, endometrial or another Lynch syndrome-related cancer before age 50. In addition, if they have a positive family history and meet the Amsterdam/Amsterdam II criteria and/or if the tumor exhibits microsatellite instability or there is loss of staining for one of the proteins expressed by the MMR genes, mutation testing is undertaken for Lynch syndrome. Testing of family members likely to carry the mutation follows. The current screening guidelines for Lynch syndrome restrict testing to those diagnosed with CRC before 50 years of age (only recently, the revised Bethesda guidelines 2004 have extended tumor MSI testing up to the age of 60 years for those that do not meet the Amsterdam criteria for family history). Therefore, most probands have an age of onset of cancer that reflects this early age. The family members or non--probands, on the other hand, are likely to have an age distribution of cancer onset that is a closer estimate of the true age at cancer onset in the Lynch syndrome population because they are less subject to selection/ascertainment bias. For example, in a recent study on a Finnish population, Hampel et al. (Gastroenterology, 2005) reported that the median age of CRC onset among non-probands was much later than among probands (61 years vs. 44 years), suggesting a need to consider Lynch syndrome in older individuals.

Methods: We analyzed 206 MMR mutation-carrying individuals (93 probands and 113 non-probands) with and without CRC, recruited at the MD Anderson Cancer Center based on the criteria described above, for age of onset to CRC using survival analysis.

Results: We found that the median age of onset of CRC was 43 years among probands but 60 years among non-probands; this difference was statistically significant ($P \leq 0.001$, log-rank test). Further, the earlier age of onset among probands as compared to non--probands was not influenced by gender, gene involved (hMLH1 or hMSH2) or the type of mutation (missense vs. truncation/deletion/insertion/other).

Conclusion: Our study replicates the findings of the Hampel study in a US population, i.e. that the median age of CRC onset may be older in Lynch syndrome than ~45 years as is presently assumed. This has implications for screening guidelines for Lynch syndrome since the current age criterion for Lynch syndrome screening may be set too low. Our findings suggest that many Lynch syndrome families who are carriers of DNA mismatch repair mutation may go undetected because they do not necessarily develop CRC at an early age.

[A24]

The Use of Early-Onset Colorectal Carcinoma as a Marker for Lynch Syndrome

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Key words: Lynch syndrome, colorectal cancer, young age

Background: Approximately eighty percent of patients with colorectal carcinoma have sporadic disease, while twenty percent most likely have a genetic component to their disease. Colorectal cancer is a rare occurrence in young individuals (less than fifty years of age) and is a major criterion for suspecting the most common autosomal dominant syndrome predisposing to colorectal carcinoma, hereditary nonpolyposis colorectal carcinoma, also known as Lynch syndrome.

Methods: Detailed personal and family histories were obtained from seventy-one patients that developed colorectal carcinoma at an age less than fifty. These patients were all treated at Rush University Medical Center in Chicago, IL between 1995 and 2005. Then, the patients were contacted by telephone, and information was obtained regarding changes in their personal and family histories since they were last treated. We were particularly interested in whether there had been a recurrence of their original diagnosis of colorectal cancer or if a different Lynch syndrome cancer had developed in them or in primary family members.

Results: 58 of the 71 (81.6%) reported no change in their personal and family histories of cancer. 4 of the 71 reported the development of a Lynch syndrome cancer, while the remaining nine reported the development of cancers that are not included in Lynch syndrome.

Conclusions: Our study suggests that early-onset colorectal carcinoma (<50 years of age) alone should not be used as a marker for Lynch syndrome, and that other criteria from the Bethesda guidelines should be met when attempting to identify families with hereditary nonpolyposis colorectal carcinoma. The relatively low frequency of development of Lynch syndrome in patients with previous colorectal carcinoma at an age less than fifty should be further confirmed with larger

sample size, population-based studies to establish more definitively whether early-onset colorectal carcinoma can be used as a predictive marker for Lynch syndrome, which may allow more effective screening in the future.

[A25]

Sensitivity of Statistical Models for the Prediction of Germline Mismatch Repair Mutations

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Background: The identification of families at risk for carrying a mutation in one of the mismatch repair genes, *MLH1*, *MSH2* or *MSH6*, has traditionally relied on Bethesda guidelines, Amsterdam and Amsterdam II Criteria, and clinical judgment. Statistical models that estimate the probability of a deleterious mutation provide quantitative tools to supplement guidelines and clinical judgment. These statistical models rely on either logistic regression (Leiden model) or a Bayesian model incorporating Mendelian probabilities and clinical information (MMRpro) to estimate the probability of a mutation. The purpose of the present study was to estimate and compare the sensitivity of the Leiden and MMRpro models among families with known mutations in mismatch repair genes.

Methods: Data from 41 independent families enrolled in the University of Michigan Cancer Genetics Registry with known pathogenic mismatch repair mutations were analyzed using two statistical models. Logistic regression models incorporated age of colorectal cancer diagnosis, fulfillment of Amsterdam criteria, and the presence of any endometrial cancer in the family. Bayesian models incorporated family structure, colorectal and endometrial cancer diagnoses and respective ages at each diagnosis. Bayesian models were run with and without microsatellite instability data. Information regarding the presence or absence of a germline mutation was assumed to be unknown for all families in Bayesian models. All calculations were performed as implemented in CancerGene at http://www4.utsouthwestern.edu/breasthealth/cagene/. Sensitivity was calculated at two thresholds for each model (mutation probabilities of ≥35%, and ≥10%).

Results: Twenty-seven families (65.8%) harbored mutations in *MSH2*, while 13 (31.7%) carried mutations in *MLH1*, and 1 family (2.4%) had a mutation in *MSH6*. At a threshold of 35% mutation probability, the sensitivity of MMRpro was 68.3%, compared to 41.5% for the Leiden model. At a threshold of 10% mutation probability, the sensitivity of MMRpro was 75.6%, compared to 73.2% for the Leiden model. Adding information about microsatellite instability increased the sensitivity of MMRpro at both thresholds (sensitivity = 70.7% and 82.9% at thresholds of 35% and 10%, respectively), and did not change the sensitivity of the Leiden model.

Conclusions: Bayesian modeling using MMRpro outperforms logistic regression modeling as implemented in the Leiden model. The sensitivity of MMRpro is especially high when incorporating information from microsatellite instability testing (83%), but still misses some families with identifiable mutations. MMRpro is a valuable aid in the identification of individuals at risk of carrying a mismatch repair mutation but is not a substitute for clinical judgment.

[A26]

Starting a New Registry: Critical Design Components

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Key words: registry, development, coordination

Background: There are many aspects to consider when starting a new research-based registry. Since its inception in 1998, coordinators of the Familial Colon Cancer Registry (FCCR) have been approached on several instances to discuss the Registry's objectives, operations, and challenges. This project involves documenting the design elements and components needed to build a successful registry.

Methods: By networking with other registries, a list of common registry objectives was compiled. These include an educational component, data and biospecimen collection, research collaboration, development, and maintenance. Each objective comprises several key elements coordinators should consider when constructing a registry.

Results: When first developing a registry, it is necessary to define the goals, establish eligibility requirements, identify funding sources, obtain institutional support, create a research study review process, and determine if it will be research or clinically focused. Once these have been identified, the data and biospecimen collection options can be more clearly defined. Frequently collected items include medical records, family history information, genetic test results, blood, and tissue samples. When data is collected, it is imperative that the information be easily extracted from the database. The ultimate goal of

a research-based registry, like the FCCR, is to facilitate translational research. In order to create mutually beneficial research collaborations, each study requesting information should pass the registry's approval process.

Once a registry has been established, many day-to--day operations must be addressed such as marketing, staffing, and working with the Institutional Review Board. Furthermore, if an institution is able to support educational components in their registry, various options may be considered including workshops, outreach presentations, newsletters, fact sheets, and having staff available to answer medical questions.

Conclusions: When starting a new research resource, it is often helpful to consult with coordinators of established registries. There are many types of registries; most have educational, clinical, and research objectives. If a registry is to function successfully and meet its objectives, careful consideration must be given to each of these components and how they interact. Registry personnel must also contribute an immense amount of foresight and organization to build a valuable resource for investigators while maintaining a positive working relationship with participants.

[A27]

Reduced mRNA stability contributes to decreased expression of the growth suppressor gene cyclin dependent kinase-2-associated protein-1 (CDK2-AP1) in microsatellite unstable (MSI) colorectal cancer (CRC)

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Key words: colorectal cancer, microsatellite instability, CDK2-AP1

Introduction: In our most recent presentation to this forum (Salt Lake City, 2006) we demonstrated that decreased cyclin dependent kinase-2-associated protein (CDK2-AP1) expression in MSI CRC is associated with a del T mutation in the poly T (8) repeat of the 3' untranslated region (UTR) of the gene. Our present study tests multiple hypotheses: H-1; The observed del T mutation in the 3' UTR of the CDK2-AP1 gene is functionally significant. H-2; Decreased CDK2-AP1 expression observed in MSI CRC and associated with the del T mutation results from reduced CDK2-AP1 mRNA stability. H-3; The del T mutation is somatic in nature.

Methods: To test the functional significance of the del T mutation (H-1) we employed a mutagenesis functional assay using a Lentiviral Vector transfer system (LV-pRRl.sin.PPT.hPGK.EGFP) to introduce both mutant and wild type poly (T) 8 3' UTR sequences into separate human CRC SW48 cell lines. Green florescent protein (GFP) expression measured transduction efficiency and surrogate CDK2-AP1 expression. CDK2-AP1 mRNA stability (H-2) was measured using a standard actinomycin D assay and the mRNA structure folding software mfold 3.2. Four human MSI CRC tumor/adjacent normal tissue pairs were sequenced for evidence of the del T mutation (H-3).

Results: H-1; Mutant (del T) samples demonstrated significantly reduced GFP expression compared to wild type GFP-3'-UTR as measured by both FACS and real--time PCR. H-2; Both the actinomycin D assay and mfold software demonstrated significantly reduced mRNA stability for the del T product compared to the wild type. H-3; Automated sequencing demonstrated the del T poly (T) 8 mutation in 4/4 human MSI CRCs. Del T was not observed in the 4 adjacent to tumor "normal" samples.

Conclusions: These results strongly support our hypotheses that the del T mutation in the poly (T) 8 repeat sequence in the 3' UTR of CDK2-AP1 is functionally significant (H-1), resulting in decreased CDK2-AP1 expression secondary to decreased mRNA stability (H-2). Importantly, the CDK2-AP1 del T mutation observed in human MSI CRC appears to be a somatic, non-germline genetic event (H-3). We conclude that these novel results significantly advance our understanding of the role of CDK2-AP1 in human MSI CRC.

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[A28]

A Lynch Syndrome Medical History Project

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Abstract submitted to Larry Rusin, MD, CGA-ICC Vice President at Lawrence.c.rusin@lahey.org

Key words: Lynch syndrome, HNPCC, MSH2

Background: A Texas family continues to exist in spite of a 50-year struggle with Lynch syndrome, an inherited condition also known as hereditary nonpolyposis colon cancer, or HNPCC. Types of cancer diagnosed within

this family include colon, uterine, thyroid, breast, kidney, melanoma, pancreas, liver, and brain. We sought to consolidate a collection of verbal and written medical history into a concise chronological timeline document to include the years 1949-2006. The psychosocial impact of the disease on this family was also observed and noted.

Methods: We will continue follow-up communications with the affected family members to update data concerning diagnosis, treatments, and outcome. The data will be presented in a timeline format coinciding with the family pedigree. Psychosocial issues discussed within family circles will be described. Finally, various methods to promote family awareness and available clinical studies will also be explored. The names of the Texas family members will remain anonymous.

Results: We found numerous cases of uterine, colon, liver, and a case of kidney and thyroid cancer among members of this family. Results of genetic testing in several members of the family revealed a mutation for Lynch syndrome (MSH2). In addition, tissue samples tested so far showed microsatellite instability.

Conclusions: Aside from a valuable family history, we hoped the data would be a resource for clinicians and scientists in addressing new hypothesis for basic and clinical investigations and educational materials. In addition, the recent scientific data documented in this project may stimulate collaborations among Lynch syndrome families and the clinical teams to develop ideas for future studies.

Recognizing the hereditary aspect of this disease and the family's efforts on prevention and early detection of Lynch syndrome cancers may enhance long-term survival and alleviate the devastating impact on the affected families.

[A29]

MLH1 germline epimutations in selected patients with early-onset nonpolyposis colorectal cancer

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Key words: germline MLH1 promoter hypermethylation, early-onset colorectal cancer, HNPCC

Background: Hereditary nonpolyposis colorectal cancer (HNPCC) is caused by germline mutations in components of the mismatch repair (MMR) system, mainly in MLH1 and MSH2 genes, and to a lesser extent in MSH6 and PMS2. Hypermethylation of CpG islands of the promoter sequence has been shown to be an important mechanism of gene silencing, and in particular of tumor suppressor genes in sporadic cancer. In the particular case of the MLH1 gene, promoter hypermethylation in colorectal tumors correlates well with loss of the MLH1 protein in sporadic MSI cases. Recently, germline epigenetic inactivation of MLH1 has been reported in a number of patients with early-onset colorectal cancer among other characteristics.

Patients and methods: From a total of 109 microsatellite instability-positive HNPCC-suspected families, eleven showed lack of MLH1 tumor expression and no germline mutations in the MMR genes. In nine of these cases and in three additional patients with multiple tumors, the study of germline MLH1 promoter hypermethylation was performed by means of MSP and COBRA. For positive cases, results were confirmed in saliva DNA and MLH1 LOH was analyzed in tumor DNA. MLH1 expression was analyzed in 24 additional colorectal tumors belonging to patients with multiple primary tumors.

Results: One of the selected patients resulted positive for the MLH1 epimutation. He had developed an epidermoid lip carcinoma and a colorectal tumor with microsatellite instability, no MLH1 expression and LOH of the gene. Parents and siblings did not carry the epigenetic alteration. None of the tumors belonging to the patients with several malignancies showed any lack of MLH1 expression.

Conclusions: We consider that screening for MLH1 germline epimutations should be performed in early--onset colorectal cancer patients with MSI, lack of MLH1 expression, and no germline mutations. In several cases the presence of several primary tumors has been observed and data suggest that this epigenetic alteration is not inheritable. In addition, the possibility of preventive treatment with demethylating agents for these patients seems very promising, taking into account the reversibility of epigenetic changes.