Investigating the Potential Role of Genetic and Epigenetic Variation of DNA Methyltransferase Genes in Hyperplastic Polyposis Syndrome

Musa Drini1,2*, Nicholas C. Wong3, Hamish S. Scott4, Jeffrey M. Craig3, Alexander Dobrovic5,6, Chelsee A. Hewitt5, Christofer Dow7, Joanne P. Young8, Mark A. Jenkins9, Richard Saffery3, Finlay A. Macrae1

1 Department of Medicine, University of Melbourne, Parkville, Victoria, Australia, 2 Department of Paediatrics, University of Melbourne, Royal Children’s Hospital, Parkville, Victoria, Australia, 3 Developmental Epigenetics, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia, 4 Department of Pathology, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia, 5 Department of Pathology, Royal Melbourne Hospital, Parkville, Victoria, Australia, 6 Department of Pathology, University of Melbourne, Parkville, Victoria, Australia, 7 Anatomical Pathology, Royal Melbourne Hospital, Parkville, Victoria, Australia, 8 Queensland Institute of Medical Research, Herston, Queensland, Australia, 9 MEGA Epidemiology, School of Population Health, University of Melbourne, Carlton, Victoria, Australia

Abstract

Background: Hyperplastic Polyposis Syndrome (HPS) is a condition associated with multiple serrated polyps, and an increased risk of colorectal cancer (CRC). At least half of CRCs arising in HPS show a CpG island methylator phenotype (CIMP), potentially linked to aberrant DNA methyltransferase (DNMT) activity. CIMP is associated with methylation of tumor suppressor genes including regulators of DNA mismatch repair (such as MLH1, MGMT), and negative regulators of Wnt signaling (such as WIF1). In this study, we investigated the potential for interaction of genetic and epigenetic variation in DNMT genes, in the aetiology of HPS.

Methods: We utilized high resolution melting (HRM) analysis to screen 45 cases with HPS for novel sequence variants in DNMT1, DNMT3A, DNMT3B, and DNMT3L. 21 polyps from 13 patients were screened for BRAF and KRAS mutations, with assessment of promoter methylation in the DNMT1, DNMT3A, DNMT3B, DNMT3L, MLH1, MGMT, and WIF1 gene promoters.

Results: No pathologic germline mutations were observed in any DNA-methyltransferase gene. However, the T allele of rs62106244 (intron 10 of DNMT1 gene) was over-represented in cases with HPS (p < 0.01) compared with population controls. The DNMT1, DNMT3A and DNMT3B promoters were unmethylated in all instances. Interestingly, the DNMT3L promoter showed low levels of methylation in polyps and normal colonic mucosa relative to matched disease free cells with methylation level negatively correlated to expression level in normal colonic tissue. DNMT3L promoter hypomethylation was more often found in polyps harbouring KRAS mutations (p = 0.0053). BRAF mutations were common (11 out of 21 polyps), whilst KRAS mutations were identified in 4 of 21 polyps.

Conclusions: Genetic or epigenetic alterations in DNMT genes do not appear to be associated with HPS, but further investigation of genetic variation at rs62106244 is justified given the high frequency of the minor allele in this case series.

Introduction

Hyperplastic Polyposis Syndrome (HPS) is a colorectal cancer (CRC) predisposition associated with the development of multiple serrated polyps, and is defined by the World Health Organization as:
(1) at least five serrated polyps proximal to the sigmoid colon with two or more of these being >10 mm; or
(2) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis; or
(3) >20 serrated polyps of any size, but distributed throughout the colon. The implied meaning of this last criterion is that the polyps are not all present in the rectum [1].

HPS is the genetic disease model for the serrated neoplasia pathway of CRC development [2–4]. This new distinct pathway of CRC is characterized by activating mutation of the BRAF proto-oncogene (specifically V600E) and widespread and concordant gene promoter hypermethylation (CpG Island methylation Phenotype or CIMP) [5–8], and is responsible for silencing of many genes by CpG island methylation in specific cancer subtypes [9]. CIMP positive CRC cancers have distinct clinico-pathological features
including proximal location, mucinous histopathology, female preponderance, and a high frequency of BRAF mutation [7,10–12]. DNA methylation disturbances are also a feature of HPS with a large proportion of BRAF mutation positive HPS polyps being CIMP positive [13,14]. Increased DNA methylation in 14 markers (MINTs 1, 2 and 3, p16, MGMT, MLH1, RASSF1, RASSF2, NORE1, RBIP, MST1, DAPK, FAS and CHIC2) in small hyperplastic polyps and in normal mucosa of patients with HPS suggests that there may be a genetic basis for this observation [6].

The aberrant DNA methylation found in the CIMP phenotype may be a consequence of a dysfunction in the machinery involved in establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for transfer of methyl groups specifically to cytosine in CpG methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15]. DNA methyltransferases (DNMTs) are a family of proteins responsible for establishing and maintaining normal DNA methylation [15].

Materials and Methods

Patient Selection

The Melbourne Health Human Research Ethics Committee approved the study (HREC2007.081) and all participants provided written informed consent to take part. We utilized the clinical database of The Royal Melbourne Hospital Bowel Cancer Surveillance Service and the Familial Cancer Clinic, to identify patients satisfying the diagnostic criteria for HPS. Detailed patient demographics, polyp number, size, location, pedigree, family and personal history of CRC and histopathology were prospectively collected (Table 1 and Table 2).

DNA sequence data on this paper have been previously reported on the GenBank database.

Polyps were reviewed by a histopathologist with an interest in the HPS (CD). The use of the term serrated polyp in this report encompasses polyps with serrated architecture and includes microvesicular hyperplastic polyps, and sessile serrated polyps with or without dysplasia. Traditional serrated adenoma and adenoma were not considered in this report. We classified lesions as advanced polyps if the size of the lesion was 10 mm or more.

Control colon tissue was obtained during colonoscopy from patients who presented with abdominal pain for investigation and completed endoscopic examination was normal.

Peripheral blood was collected for germline mutation analysis and immortalized EBV transformed lymphoblast cell lines (LCLs) were generated. If surveillance colonoscopy was performed, polyp tissue was collected and stored in RNALater (Ambion-Applied Biosystems).

| Cases | HPs location | Classification of polyps | Size | BRAF/KRAS mutation |
|-------|--------------|--------------------------|------|--------------------|
| 1     | Sigmoid colon| SSA                      | 10 mm| KRAS               |
| 2     | Descending colon| SSA                  | 8 mm | WT                 |
| 3     | Rectum       | MVHP                     | 5 mm | KRAS               |
| 4     | Sigmoid colon| MVHP-SSA                 | 9 mm | BRAF               |
| 5     | Transverse colon| MVHP                 | 6 mm | KRAS               |
| 6     | Descending colon| HP                    | 9 mm | BRAF               |
| 7     | Ascending colon| SSA                    | 7 mm | BRAF               |
| 8     | Rectum       | SSA                      | 5 mm | BRAF               |
| 9     | Ascending colon| SSA                   | 12 mm| WT                 |
| 10    | Rectum       | SSA                      | 4 mm | BRAF               |
| 11    | Transverse colon| Mixed HP/SSA          | 6 mm | WT                 |
| 12    | Transverse colon| SSA                    | 8 mm | BRAF               |
| 13    | Rectum       | SSA                      | 5 mm | WT                 |
| 14    | Sigmoid colon| HP                       | 5 mm | WT                 |

Total DNA extraction

LCLs (lymphoblast cell lines) were available from 45 patients with HPS. DNA was extracted from all cases using DNeasy 96 Blood and Tissue kits according to the manufacturer’s instructions (Qiagen, Hilden, Germany). In addition, DNA was extracted from...
21 polyps obtained from 13 different HPS cases. DNA concentration and quality was assessed by absorbance spectrophotometry and agarose gel electrophoresis.

RNA Isolation and Real-Time Reverse-Transcription PCR
Total RNA was isolated using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions, followed by treatment with Turbo DNA-free (Ambion/Applied Biosystems, Austin, TX, USA) to remove contaminating genomic DNA. Reverse transcription using random hexamers was performed with SuperScript VILO cDNA Synthesis Kit (Invitrogen). To determine expression levels of genes of interest, quantitative RT-PCR using SYBR Green-based analysis and Master Mix (Invitrogen) was carried out with 10 μM sense and antisense primers (primers on request). Reactions were performed in triplicate and analysed using an ABI 7300 Sequence Detection System (Applied Biosystems). Relative expression levels were determined using the standard ΔCt method with GAPDH housekeeping gene used for normalisation.

Germline mutation detection
We employed High Resolution Melting (HRM) analysis as a novel and cost effective method to search for the presence of unknown variants in DNMT genes, from 45 cases with available genomic DNA. DNA was first amplified by real time PCR in the presence of LightCycler® 480 High Resolution Melting Dye using a LightCycler® 480 System (Roche Applied Sciences). After the PCR, the successive melting curve experiment was performed in the same apparatus. Gene scanning software was used to identify sequence variation by melt profiling, PCR and DNA melting were performed in triplicate. PCR was optimized with titration of MgCl2 and variation in annealing temperatures. In brief, 10 ng genomic DNA was used in the PCR, Master mix (Roche Applied Sciences), and final forward and reverse primer (concentration 0.2 μM). Sense and antisense PCR primers for DNMT genes were designed using consensus coding sequences: DNMT1 (CCDS12228.1), DNMT3A (CCDS1718.1), DNMT3B (CCDS13294.1) and DNMT3L (CCDS13705) [34]. When amplicon length exceeded 300 bp or nonspecific product was evident following electrophoresis on a 2% agarose gel, we designed alternative primer pairs using the Primer3 software package (http://bioapps.tulane.edu/bioapps/primer3_www.cgi). The online POLAND program was used to confirm that amplicons contained a single melting domain [35].

Somatic mutation detection for BRAF and KRAS
High resolution melting (HRM) analysis was performed using a LightCycler® 480 (Roche Diagnostics, Penzberg, Germany) in a modification of the previously published method of [36]. The reaction mixture contained: 1x PCR buffer, DNA template, 200 μM dNTPs (Fisher Biotech Australia, Wembley, Western Australia), 5 μM SYTO® 9 (Invitrogen, Carlsbad, CA), and 0.5 U HotStarTaq polymerase (Qiagen, Germantown, MD, USA). Various concentrations of MgCl2 and primers (primers on request) were used specific to each assay in a 10 μl final reaction volume. PCR reactions were performed in triplicate.

Quantitative DNA methylation analysis by MALDI-TOF MassARRAY EPITYPING and bisulphite sequencing
DNA was extracted from 21 polyps (13 cases with HPS) and 5 controls (colonic mucosa from non-HPS cases) using the DNeasy kit (Qiagen). Bisulphite conversion was performed using the MethylEasy Xceed kit according to manufacturer’s instructions (Human Genetic Signatures, Sydney, Australia). 1–2 μg of genomic DNA was used for bisulphite conversion. The converted DNA was eluted to a final concentration of 20 ng/μl. 20 ng of converted DNA was used for PCR amplification.

We employed SEQUENOM EpiTYPER analysis [37] for detection and quantitation of DNA methylation of the promoter regions of DNMT1, DNMT3A, DNMT3B and DNMT3L. In brief, genomic DNA was bisulphite treated using MethylEasy Xceed kit (Human Genetic Signatures), PCR amplification was performed using primers directed to the promoter regions of the DNMT genes (primers on request). Amplicons were then subjected to the EpiTYPER chemistry. Brieﬂy, amplicons were treated with shrimp alkaline phosphatase treatment followed by in vitro transcription and base speciﬁc cleavage (SEQUENOM, San Diego, CA). Samples were then analysed by MALDI-TOF mass spectroscopy and the methylation ratios obtained using EpiTYPER v1.0.5 software (SEQUENOM). Further analysis and cleaning of data was performed using the R statistical package (http://www.cran.org) and scripts developed in house. This included identiﬁcation and removal of CpG units that overlapped with other peaks following mass spectroscopy. Further data cleaning and curation was then performed in R to remove CpG units that did not yield data in at least 70% of samples. Samples, from which less than 40% of CpG units yielded data, were also not included in subsequent analyses.

The DNMT3L amplicons were subjected to cloning and DNA sequencing as outlined in [38]. Amplicons were cloned into pGEMT-Easy Vector (Promega, Madison, WI, USA) and then transformed into DH5α E. coli. Positive clones were selected for automatic DNA sequencing performed by the Australian Genome Research Facility (AGRF, Melbourne, Australia). Sequencing data was then analysed using BiQ-Analyzer [39].

Statistical analysis
After data cleaning and curation of SEQUENOM assays, data was analysed using the Heatmap.2 function in R whereby unsupervised hierarchal clustering of the samples and CpG units was performed. Dendrograms and associated heatmaps were generated according to the DNA methylation ratio of specific CpG units and samples analysed. Geometric means methylation levels were calculated for each tissue type, prior to statistical analysis using a Student’s t-test. A box and whisker plot was generated in R to display the distribution of DNA methylation ratios between polyp and germline tissues across the DNMT3L promoter. Calculations were performed to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the associations between allele frequency and disease. All statistical tests were two-sided and P-value, 0.05 was considered as a significant level of statistical evidence to reject the null hypothesis. All statistical analyses were done using Stata 10.0.

Results
DNA methyltransferase exon scanning by High Resolution Melting (HRM) Analysis
In order to ascertain whether the previously reported methylation disruption associated with HPS may be due to genetic variability in DNMTs, we screened a total of 92 DNMT exons for novel mutations by HRM analysis in LCL genomic DNA from HPS subjects. This comprised 40 exons from DNMT1, 17 from DNMT3A, 24 from DNMT3B and 11 from DNMT3L. We found three previously identified single nucleotide polymorphisms (SNPs) in DNMT1 (synonymous rs721186 and rs2228015) and intronic (rs62106244) and one SNP in DNMT3A (synonymous rs2276598) (Table 3).
The T allele of the rs62106244 C>T variant (Fig. 1) was present in 7 out of 45 cases all of whom were heterozygous. To establish the frequency of this uncharacterised variant in the Caucasian population generally, we scanned 300 control samples, and found 16 that were heterozygous at this site. Chi-square analysis confirmed a statistically significant over representation of both the CT genotype and T allele in HPS cases ($\chi^2 = 6.66$, $p = 0.01$; $\chi^2 = 7.45$, $p<0.01$ respectively). We observed that cases with HPS were approximately three times more likely to carry the variant (15.6%) compared to controls (5.3%), and this difference was statistically significant ($p = 0.01$). However, given the rarity of the variant the confidence intervals were wide (relative risk = 2.9; 95% confidence interval 1.1 to 6.5). DNMT3B and DNMT3L exon scanning did not reveal any SNPs in our HPS cases.

Quantitative methylation analysis of CRC CIMP-associated genes and DNMTs

Previous studies using a small number of patients with HPS and methylation specific PCR (MSP) have identified elevated levels of specific promoter methylation in both polyps and normal mucosa relative to non-diseased mucosa from subjects with sporadic serrated polyps [6]. This includes methylation of the MGMT and MLH1 genes. In this study, we measured DNA methylation of the IGF2 differentially methylated region (DMR), H19, MGMT, MLH1 and WIF1 promoters. Contrary to previous reports [40]; [13,14] we found that, MGMT and MLH1 were unmethylated (Fig. S1) in both polyps and matched normal mucosa, while there was no significant difference in DNA methylation of IGF2, H19, and WIF1 (Fig. S1) between polyp and disease free tissue.

In addition to examining CIMP markers, we also examined the potential for methylation based dysregulation of the DNMT genes themselves. Little data is available regarding methylation status of this family of genes in non-diseased somatic tissue, and only limited studies have examined this in disease [41,42]. We studied DNA methylation of the promoter region of the DNMT1, -3A and -3B genes in HPS cases, comparing polyp-derived DNA, normal mucosa from the same patient (where available), and mucosa from controls with no disease. All three promoter regions were generally unmethylated in both polyp and normal mucosal tissue, with less than 10% methylation detected by SEQUENOM EpiTYPER (data not shown). We also found no evidence of promoter methylation of these genes in LCL genomic DNA (data not shown).

Interestingly, we found that DNMT3L was hypomethylated in both normal gut mucosa (mean methylation 0.33, SD 0.24, n = 12) and polyp tissue (mean methylation 0.36, SD 0.25, n = 21) relative to matched LCL DNA (mean 0.57, SD 0.24 n = 19); see unsupervised clustering figure 2A and box and whisker plot figure 2B). This difference was highly significant ($p = 5.1 \times 10^{-9}$ Student’s t-test) and was confirmed by bisulphite sequencing (Fig. 3). We were unable to test expression level directly in HPS samples due to a lack of RNA, however a negative correlation between mean methylation and expression level was found in normal colonic tissue ($R = -0.64$; Fig. 4).

KRAS mutation may correlate with DNMT3L promoter methylation

CIMP and BRAF mutation are hallmarks of serrated tumours and BRAF V600E has been proposed as an important biological marker for HPS specific cancers [45]. We performed BRAF (V600E) and KRAS screening with HRM on DNA from serrated polyps derived from HPS cases and controls. Consistent with previous published data [7,44] we found that the BRAF V600E mutation was common in serrated polyps (11 out of 21) whereas the KRAS mutation was evident in only 4 out of 21. KRAS mutations were found primarily in polyps located in the transverse colon (two) and rectum (two). Interestingly one patient had two polyps with the BRAF somatic mutation and one with the KRAS somatic mutation, all within the right colon from the same patient. Examination of the level of DNMT3L promoter methylation and KRAS mutation-containing polyps revealed an association between these distinct genetic and epigenetic modifications (Table 4).

Polyps with KRAS mutations were far more likely to show DNMT3L promoter methylation at levels similar to those found in LCLs ($p = 0.0053$). In contrast, polyps with the BRAF mutation did not show any significant association with DNMT3L promoter methylation.

Table 3. SNPs identified with HRM on germline DNA.

| Gene   | SNP ID  | Exon/Intron | DNA change | Clinical association |
|--------|---------|-------------|------------|----------------------|
| DNMT1  | rs721186| Exon        | C/T        | Unknown              |
| DNMT7  | rs62106244| Intron     | C/T        | Unknown              |
| DNMT3A | rs2228613| Exon        | A/C        | Unknown              |
| DNMT3A | rs2276598| Exon        | C/T        | Unknown              |

**Figure 1. Normalized and temp-shifted difference plot for DNMT1 gene SNP rs62106244.** Seven cases were heterozygous for rs62106244. The top arrow shows the sequence of the DNMT1 gene. doi:10.1371/journal.pone.0016831.g001
Discussion

Given the significant association of HPS and associated tumours with increasing levels of aberrant promoter methylation (CIMP), including extensive methylation in the normal mucosa, and the role of DNMTs in both the establishment and maintenance of DNA methylation in humans, we examined the potential association of genetic and epigenetic disruption of DNMTs in HPS.

Genetic variation is the DNMT1 gene is potentially associated with HPS

Variants in DNMT1 have been identified as risk factors for disease including systemic lupus erythematosus [45]. Genetic deficiency of DNMT3B causes ICF syndrome, a recessive human disorder characterised by immunodeficiency, centromere instability, and facial anomalies [46]. Variants in other DNMTs (i.e. DNMT3L, DNMT1) have been associated with human cancers [47–50]. Recently, a rare variant of DNMT3L was specifically associated with reduced methylation of sub-telomeric regions in humans [51]. In this study we demonstrated that HPS is not
Table 4. Quantitative methylation (%) of DNMT gene promoters: 21 serrated polyps stratified by BRAF and KRAS mutation.

|                          | Mean DNMT1 methylation (%) | Mean DNMT3A methylation (%) | Mean DNMT3B methylation (%) | Mean DNMT3L methylation (%) |
|--------------------------|----------------------------|-----------------------------|-----------------------------|----------------------------|
| Polyps with BRAF mutation| 3.3^                         | 4.3^                        | 21^                          | 37^                        |
| Polyps with KRAS mutation| 2.8^                        | 4.1^                        | 27^                          | 55^^                       |
| Polyps WT for BRAF and KRAS| 3.6                         | 4.4                         | 25                           | 30                         |

^p-value < 0.05 for DNMT1, DNMT3A and DNMT3B.
*p-value = 0.147 (BRAF/WT).
**p-value = 0.0053 (KRAS/WT).

recently the loss of DNA methylation at the DNMT3L promoter was found to be a positive biomarker for cervical cancer [60]. DNMT3L over expression in cervical cancer cells increases cellular proliferation, anchorage independent growth and nuclear reprogramming of cells, all central events in tumour development [61].

An interaction between DNMT3L methylation and KRAS mutation?

Previous studies that have examined the relationship between BRAF and KRAS mutations in a wide range of colonic polyp and cancer tissue have found a mutually exclusive distribution of these mutations [7]. DNMT3L methylation percentage across all CpG sites analysed for cases with BRAF and KRAS mutation revealed increased methylation of the DNMT3L promoter in cases with a KRAS mutation when compared with polyps with the normal variant (p = 0.0053), however due to small number of cases this observation need to be investigated in a larger number of polyps harbouring KRAS mutation. Additionally we found both BRAF and KRAS mutations in a single case (two polyps harbouring BRAF mutation and one polyp with KRAS mutation), which, though relatively rare, has been previously described [44]. Lesions harbouring KRAS mutations have been associated with distal location within the colorectum [62] MSI-L status [63] and unfavourable prognosis [64]. Our findings with regard to DNMT3L methylation and KRAS mutation need to be investigated further as recently aberrant promoter hypomethylation of DNMT3L has been linked with cervical cancer tumorigenesis [60]. The increased DNMT3L methylation seen in KRAS mutated
polyp is commensurate with the lower levels of methylation generally seen in these polyps relative to those with BRAF mutations.

Conclusions

To date, there is no single genetic abnormality known to underlie HPS. In addition, factors determining the transition to colon carcinoma remain unknown. There have been several reports implicating DNMT dysregulation in carcinogenesis [41,65]. However, the degree to which DNMTs may contribute to the development of precancerous lesions remains poorly understood. This study represents the first investigation of the possible role of the DNMT family of genes in the development of HPS. The data generated do not exclude a functional role of DNMT dysregulation in the development of HPS and associated disorders, however no exonic germline mutations were discovered, suggesting that regulation of DNMTs may occur via alternative mechanisms in this condition. It is interesting to speculate that the observed hypomethylation of the DNMT3L promoter in normal gut mucosa in the HPS patients identified here, may also play a role in the aberrant de novo establishment of tumour suppressor methylation seen in most CRC.

Supporting Information

Figure S1 Quantitative methylation analysis (% with SEQUENCE-NOM: genes analysed H19, MGMT, MLH1, WIF1, BRAF and KRAS. Samples: S1, S13, S10, S11 and S26 were disease free tissue. (TIIF)

Acknowledgments

We thank Dr Justine Ellis and Dr Lavinia Gordon for help with statistical analysis.

Author Contributions

Conceived and designed the experiments: MD NCW RS HSS FAM. Performed the experiments: MD NCW RS HSS AD JMC FAM AD. Made polyps histopathology assessment: CD. Contributed on biostatistics with regard to data analysis and critical review of manuscript: MAJ.

References

1. Burt R, Jass JR (2000) Hyperplastic Polyposis In: Hamilton SR, Aulton LA, eds.: Pathology and Genetics of Tumours of Digestive System. Lyon: IARC Press. pp 135–136.
2. Jass JR (2000) Serrated adenoma of the colorectum and the DNA-methyltransferase phenotype. Nat Clin Pract Oncol 2(8): 398–405.
3. Sutter DC, Jass JR, Fenoglio-Preiser C, Batts KP (2005) Serrated polyps of the large intestine: a morphologic and molecular review of an evolving concept. Am J Clin Pathol 124(3): 380–91.
4. Goldstein NS (2006) Serrated pathway and APC (conventional-type) colorectal polyps: molecular-morphologic correlations, genetic pathways, and implications for classification. Am J Clin Pathol 125(1): 146–53.
5. Weisenberger DJ, Siegmund KD, Campan M, Young Jl, Long TI, et al. (2006) CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 38(5): 787–93.
6. Minos P, Baker K, Grossani R, Chong G, Foulkes WD, et al. (2006) Extensive DNA methylation in normal colorectal mucosa in hyperplastic polyposis. Gut 55(10): 1467–74.
7. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, et al. (2004) Characterization of colorectal cancers showing hypermethylation at multiple Cpg islands. Gut 51(6): 797–802.
8. Toyota M, Ohe-Toyota M, Fujinaga M, et al. (2001) CpG Island methylator phenotype and K-ras mutation to location and histologic subtype. Cancer Res 61(7): 2676–81.
9. Chao AG, Issa JP, Morris JS, Hamilton SR, Rashid A (2002) Concordant CpG island methylation in hyperplastic polyposis. Am J Pathol 160(2): 529–36.
10. Issa JP, Shen L, Toyota M (2005) CIMP, at last. Gastroenterology 129(5): 1121–4.
11. Jones PA, Basilion SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genetics 3(6): 415–426.
12. Frigola J, Song J, Sztirzaker C, Mihalceava RD, Peinado MA, et al. (2006) Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band. Nature Genetics 38(5): 540–549.
13. Bestor TH (1998) GENETOGENESIS ’98: Methylation and the Unequal Developmental Potentials of the Oocyte and Sperm Genomes. Am J Hum Genet 62: 1269–1273.
14. Chan AO, Issa JP, Morris JS, Hamilton SR, Rashid A (2002) Concordant CpG island methylation in hyperplastic polyposis. Am J Pathol 160(2): 529–36.
15. Jones PA, Basilion SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genetics 3(6): 415–426.
16. Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genetics 3(6): 415–428.
17. Oka M, Xie S, Li E (1989) Dnmt2 is not required for de novo and maintenance methylation of viral DNA in embryonic stem cells. Nucleic Acid Research 26: 2536–2540.
18. Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99(5): 247–57.
19. Xie S, Wang Z, Okano M, Nogami M, Li Y, et al. (1999) Clooning, expression and chromosomal locations of the human DNM3 gene family. Gene 236(1): 87–95.
20. Apolll U, Kawasaki K, Scott HS, Oliffa J, Vihinen M, et al. (2000) Isolation and initial characterization of a novel zinc finger gene, DNM3L, on 2q12-23, related to the cytosine-5-methyltransferase 3 gene family. Genomics 65(3): 293–8.
21. Webster K, O'Bryan MK, Fletcher S, Creweher PE, Aapola U, et al. (2005) Mutic and epigenetic defects in Dnm3l knockout mouse spermatogenesis. PNAS 102(11): 4068–4073.
22. Guenbaunum Y, Cedar H, Kazin A (1982) Substrate and sequence specificity of a ratiosio DNA methylase. Nature 295: 620–22.
23. flynn J, Glickman JF, Reich NO (1996) Murine DNA cytosine-C5 methyltransferase: pre-steady- and steady-state kinetic analysis with regulatory DNA sequences. Biochemistry 35(23): 7308–15.
24. Kaneda M, Okano M, Hata K, Sado T, Tsuchimoto N, et al. (2004) Essential role for the de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. Nature 429(6994): 900–3.
25. Dodge JE, Okano M, Dick F, Tsuchimoto N, Chen T, et al. (2005) Inactivation of Dnm3b in mouse embryonic fibroblasts results in DNA hypomethylation, chromosomal instability, and spontaneous immortalization. J Biol Chem 280(18): 17986–91.
26. Aapola U, Lyle R, Krohn K, Antonarakis SE, Peterson P (2001) - Isolation and initial characterization of the mouse Dnmt3 gene. - Cytogeten Cell Genet 92(1-2): 122–6.
27. Jia D, Jurkowska RZ, Zhang X, Jeltsch A, Cheng X (2007) Structure of Dnmt3a bound to Dnmt3l suggests a model for de novo DNA methylation. Nature 449(7159): 248–51.
28. Kanai Y, Hirohashi S (2007) Alterations of DNA methylation associated with abnormalities of DNA methyltransferases in human cancers during transition from a precancerous to a malignant state. Carcinogenesis 28(12): 2434–42.
29. Issa JP (2004) CpG island methylator phenotype in cancer. Nat Rev Cancer 4(12): 988–93.
30. Nosho K, Shima K, Iihara N, Kure S, Baba Y, et al. (2009) DNMT3B expression might contribute to CpG island methylator phenotype in colorectal cancer. Clin Cancer Res 15(11): 3663–71.
31. Ibrahim AE, Azevedo MJ, Silva AI, Wylie AH, Greger L, et al. (2010) Sequential DNA methylation changes are associated with DNMT3B overexpression in colorectal neoplasia progression. Gut 223602 Published Online.
32. sjolomn T, Jones S, Wood LD (2006) The consensus coding sequences of human breast and colorectal cancers. Science 314: 260–274.
33. Steger G (1994) Thermal denaturation of double-stranded nucleic acids: prediction of temperatures critical for gradient gel electrophoresis and polymerase chain reaction. Nucleic Acid Research 22(14): 2760–2768.
36. Krypyay M, Newham GM, Thomas DA, Conron M, Dobrovic A (2006) High resolution melting analysis for the rapid and sensitive detection of mutations in clinical samples: KRAS codon 12 and 13 mutations in non-small cell lung cancer. BMC Cancer 6:295.

37. Elrich M, Correll D, van den Boom D (2006) Introduction to EpIQPER for Quantitative DNA Methylation Analysis Using the MassARRAY System. Sequenom.

38. Wong N, Morley R, Saffery R, Craig J (2008) Archived Guthrie blood spots as a novel source for quantitative DNA methylation analysis. Biotechniques 45(4): 423–4.

39. Bock C, Reither S, Mikeska T, Pahlken M, Walter T, et al. (2005) BiQ Analyzer: visualization and quality control for DNA methylation data from bisulfite sequencing. Bioinformatics 21(21): 4067–4068.

40. Yang S, Farraye FA, Mack C, Poulik O, O’Brien MJ (2004) BRAF and KRAS Mutations in hyperplastic polyps and serrated adenomas of the colorectum: relationship to histology and CpG island methylation status. Am J Surg Pathol 28(11): 1425–9.

41. Roll JD, Rivenbark AG, Jones WD, Coleman WB (2008) DNMT3b overexpression contributes to a hypermethylator phenotype in human breast cancer cell lines. Mol Cancer 7: 15.

42. Jin B, Tao Q, Peng J, Soo HM, Wu W, et al. (2008) DNA methyltransferase 3B (DNMT3B) mutations in ICF syndrome lead to altered epigenetic modifications and aberrant expression of genes regulating development, neurogenesis and immune function. Hum Mol Genet 17(3): 690–709.

43. Minoo P, Moyer M, Jass J (2007) Role of BRAF-V600E in the serrated pathway of colorectal tumourigenesis. J Pathol 212(2): 124–133.

44. Carvajal-Carmona L, Howarth K, Lockett M, Polanco-Echeverry G, Volkos E, et al. (2007) Molecular classification and genetic pathways in hyperplastic polyposis syndrome. J Pathol 212: 378–385.

45. Park B, Kim LH, Shin HD, Park YW, Uhm WS, et al. (2004) Association analysis of DNA methyltransferase-1 (DNMT1) polymorphism with systemic lupus erythematosus. J Hum Genet 49(11): 642–6.

46. Hansen RS, Wijmenga C, Luo P, Stanek AM, Canfield CM, et al. (1999) The DNMT3L overexpression mimics carcinogenesis. Epigenetics 4(5): 322–9.

47. Kato Y, Kaneda M, Hata K, Kumai K, Hisano M, et al. (2007) Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. Hum Mol Genet 16(19): 2272–80.

48. Bourc’his D, Xu GL, Lin CS, Bollman B, Restor TH (2001) Dnmt3L and the establishment of maternal genomic imprints. Science 294: 2536–2539.

49. Ushijima T, Watanabe N, Shimizu K, Miyamoto K, Sugimura T, et al. (2009) Aberrant DNA methylation on chromosome 16 is an early event in hepatocarcinogenesis. Japanese journal of cancer research 87(12): 1210–1217.

50. Ushijima T, Watanabe N, Shimizu K, Miyamoto K, Sugimura T, et al. (2005) Detection and interpretation of altered methylation patterns in cancer cells. Nat Rev Cancer 5(3): 223–231.

51. El-Maarri O, Karetta MS, Mikeska T, Becker T, Diaz-Lacava A, et al. (2009) A systematic search for DNA methyltransferase polymorphisms reveals a rare DNMT3L variant associated with subtelomeric hypomethylation. Hum Mol Genet 26: 26.

52. Petra M, Lin X, Salzberg SL (2001) GeneSplicer: a new computational method for splice site prediction. Nucleic Acid Res 29(5): 1185–90.

53. Anundsdottir L, Kraft P, Stolzenberg-Solomon RZ, Fuchs CS, Petersen GM, et al. (2009) Genoma-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. Nat Genet 41(9): 986–90.

54. Hindorff L, Sethupathy P, Junkins H, Ramos EM, Mehta JP, et al. (2009) Potential etiologic and functional implications of genome-wide association loci for human disease and traits. Proc Natl Acad Sci 106(23): 9362–7.

55. Hermann JG, Urban A, Polyak K, Graff JR, Ahuja N, et al. (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci 95(12): 6870–5.

56. Boss JL (1987) Prevalence of ras gene mutations in human colorectal cancers. J Pathol 152(2): 145–9.

57. Kato Y, Kaneda M, Hata K, Kumai K, Hisano M, et al. (2007) Alteration of hMLH1 promoter hypermethylation in colorectal cancer cell lines. J Pathol 212: 378–385.

58. Kanai Y, Ushijima S, Tsuda H, Sakamoto M, Sugimura T, et al. (1996) Methylation Profile at the DNMT3L Promoter: A Potential Biomarker for Cervical Cancer. Epigenetics 2(2): 80–85.

59. Gokul G, Ramakrishna G, Khosla S (2009) Reprogramin of HeLa cells upon Dnmt3L variant associated with subtelomeric hypomethylation. Hum Mol Genet 26: 26.

60. Bross JL (1976) Prevalence of ras gene mutations in human colorectal cancers. Nature 237: 293–297.

61. Jass JR, Bieden KG, Cunningham MC, Simms LA, Walsh M, et al. (1999) Characterization of a subtype of colorectal cancer combining features of the suppressor and mild mutator pathways. J Clin Pathol 52(6): 455–60.

62. Benhattar J, Losi L, Chaubert P, Givel JC, Costa L (1993) Prognostic significance of K-ras mutations in colorectal carcinoma. Gastroenterology 104(327): 293–297.

63. Lin RK, Hsu HS, Chang JW, Chen CY, Chen JT, et al. (2007) Alteration of DNA methyltransferases contributes to 5’CpG methylation and poor prognosis in lung cancer. Lung Cancer 55: 205–213.

64. Chou E, Lipton L, Lynch E, D’Souza R, Aragona C, et al. (2006) Hyperplastic polyposis syndrome: phenotypic presentations and the role of MBDD4 and MYH. Gastroenterology 131(1): 30–9.
Author/s:
Drini, M; Wong, NC; Scott, HS; Craig, JM; Dobrovic, A; Hewitt, CA; Dow, C; Young, JP; Jenkins, MA; Saffery, R; Macrae, FA

Title:
Investigating the Potential Role of Genetic and Epigenetic Variation of DNA Methyltransferase Genes in Hyperplastic Polyposis Syndrome

Date:
2011-02-10

Citation:
Drini, M., Wong, N. C., Scott, H. S., Craig, J. M., Dobrovic, A., Hewitt, C. A., Dow, C., Young, J. P., Jenkins, M. A., Saffery, R. & Macrae, F. A. (2011). Investigating the Potential Role of Genetic and Epigenetic Variation of DNA Methyltransferase Genes in Hyperplastic Polyposis Syndrome. PLOS ONE, 6 (2), https://doi.org/10.1371/journal.pone.0016831.

Persistent Link:
http://hdl.handle.net/11343/263937

File Description:
Published version

License:
CC BY