Human metastable epiallele candidates link to common disorders

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Metastable epialleles (MEs) are mammalian genomic loci where epigenetic patterning occurs before gastrulation in a stochastic fashion leading to systematic interindividual variation within one species. Importantly, periconceptual nutritional influences may modulate the establishment of epigenetic changes, such as DNA methylation at MEs. Based on these characteristics, we exploited Infinium HumanMethylation450 BeadChip kits in a 2-tissue parallel screen on peripheral blood leukocyte and colonic mucosal DNA from 10 children without identifiable large intestinal disease. This approach led to the delineation of 1776 CpG sites meeting our criteria for MEs, which associated with 1,013 genes. The list of ME candidates exhibited overlaps with recently identified human genes (including *CYP2E1* and *MGMT*, where methylation has been associated with Parkinson disease and glioblastoma, respectively) in which perinatal DNA methylation levels where linked to maternal periconceptual nutrition. One hundred and eighteen (11.6%) of the ME candidates overlapped with genes where DNA methylation correlated (r > 0.871; p < 0.055) with expression in the colon mucosa of 5 independent control children. Genes involved in homophilic cell adhesion (including cadherin-associated genes) and developmental processes were significantly overrepresented in association with MEs. Additional filtering of gene expression-correlated MEs defined 35 genes, associated with 2 or more CpG sites within a 10 kb genomic region, fulfilling the ME criteria. DNA methylation changes at a number of these genes have been linked to various forms of human disease, including cancers, such as asthma and acute myeloid leukemia (*ALOX12*), gastric cancer (*EBF3*), breast cancer (*NAV1*), colon cancer and acute lymphoid leukemia (*KCNK15*), Wilms tumor (protocadherin gene cluster; *PCDHAs*) and colorectal cancer (*TCERG1L*), suggesting a potential etiologic role for MEs in tumorigenesis and underscoring the possible developmental origins of these malignancies. The presented compendium of ME candidates may accelerate our understanding of the epigenetic origins of common human disorders.

Introduction

Epigenetic changes, including DNA methylation and histone modification, can influence gene expression independently from the genetic code. These tightly regulated mechanisms play an essential role in mammalian differentiation and phenotype development. As an exception to tight regulation, epigenetic modifications at metastable epialleles (MEs) occur stochastically during early embryonic development, leading to systematic (affecting all tissues of the body) and prominent interindividual variation. DNA methylation at MEs can be nutritionally responsive, which implicates MEs in the developmental origins of common human disorders.[1,2] Therefore, MEs are prime examples of environmental epigenetics.[3]

A 2-tissue parallel epigenomic screen on peripheral blood leukocyte (PBL) and hair follicle DNA indicated the existence of MEs in humans.[1] However, the microarrays used in that study were of low coverage and did not allow for the quantitative estimation of DNA methylation at ME candidates. Potential associations between the putative MEs and transcription were not examined

either. Here, we exploited Infinium HumanMethylation450 BeadChip kits in a 2-tissue parallel screen. These arrays can support an accurate, quantitative determination of DNA methylation at select CpG sites both from PBL (mesodermal origin)[4,5] and colonic mucosal DNA (endodermal origin).[6] Association between the novel ME candidates and gene expression was examined in an independent cohort.

Results

ME candidates are relevant for homotypic cell adhesion. Microarray data was analyzed from 10 control children (pediatric patients with gastrointestinal complaints, but in whom the endoscopic—histologically normal intestinal mucosa—and clinico-laboratory investigations did not reveal abnormal organic etiology for their complaints; see Materials and Methods and **Table S1A**). These patients had both PBL[4] and colonic mucosal DNA samples examined (data: www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32148). Our earlier publication on the PBL samples showed significant correlation between

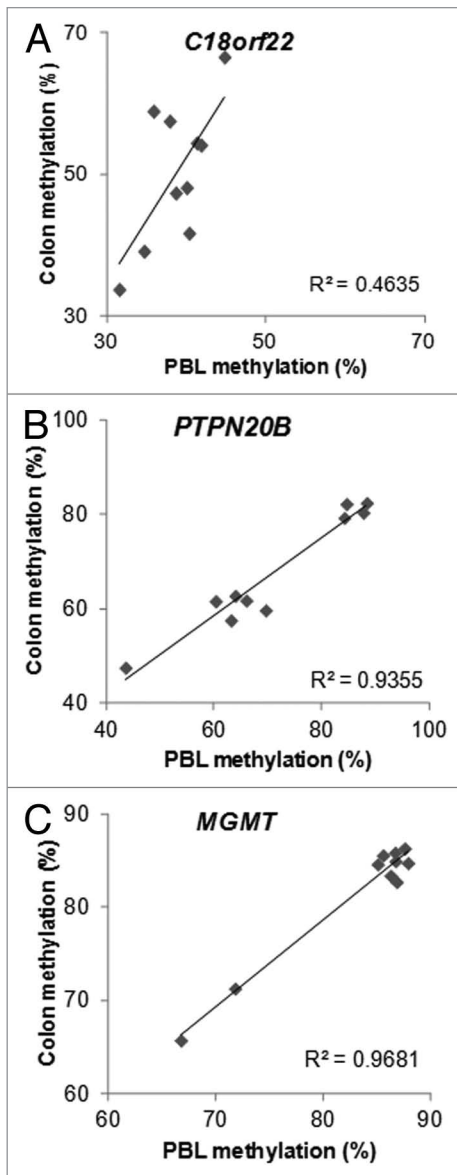*Correspondence to: Richard Kellermayer; Email: kellerma@bcm.edu

Figure 1. Metastable epiallele (ME) candidates, where epigenetic responsiveness to periconceptual maternal micronutrient supplementation was observed in the cord blood of male (**A–B**) and female (**C**) newborns (see text for details). DNA methylation from peripheral blood (mesodermal origin) DNA, and colonic mucosa (colon, endodermal origin) showed significant intraindividual correlation, but > 10% interindividual methylation differences, n = 10. *C18orf22*: r = 0.66, p = 0.038; *PTPN20B*: r = 0.97, p < 0.001; *MGMT*: r = 0.98, p < 0.001.

DNA methylation levels and β values by bisulfite pyrosequencing validation of the arrays.[4] Similarly, a significant correlation was found between microarray and pyrosequencing results in the colonic mucosa of 8 controls at 5 independent loci in this work (p = 0.0002; **Fig. S1**; see **Table S2** for primer sequences).

A total of 1,776 CpG sites met our criteria for MEs, which associated with 1,013 genes (**Table S3**). Importantly, *ZFYVE28*, one of the five earlier validated human MEs,[1] was among this list, in spite of the different tissues studied and the dissimilar molecular methods employed. For ontology analyses, the ME associated genes were compared with the rest of the genome, since the array covers 99% of known human genes. Remarkably, Interpro (www.ebi.ac.uk/interpro) comparisons revealed that cadherin, N-terminal (IPR:013164, 8.7-fold) and cadherin (IPR:002126, 5.12-fold), genes in which DNA methylation has been linked to metastasis in multiple forms of cancer,[7,8] were significantly (p < 0.05) overrepresented, while the conserved protein category of olfactory receptor (IPR:000725, 0.14-fold) was underrepresented in connection with MEs (**Table S4A**). Cadherins play an important role in homotypic cell adhesion.[9] Our finding that MEs are overrepresented in association with these genes indicates that periconceptual environmental influences may be important in modifying cell adhesion in humans. On the contrary, epigenetic development at olfactory receptors may be protected against early prenatal environmental changes.

MEs overlap with genes epigenetically responsive to periconceptual nutrition. A recent prospective human study found that periconceptual maternal micronutrient supplementation induced gender-specific DNA methylation changes that could be detected from cord blood.[10] Twenty-one female genes and 14 male genes were observed to have associations between DNA methylation and periconceptual dietary supplementation. Importantly, 2 of the male genes (14.3%) and 2 of the female genes (9.5%) had overlap with our candidate ME list. In addition, there were no such associations with the control list of 1,399 genes, in which DNA methylation variation did not correlate (r < 0.5; p > 0.14) between PBL and colonic mucosa (Chi square p = 0.04). Namely, *C18orf22* (*RBFA*, **Fig. 1A**) and *PTPN20B* (**Fig. 1B**) overlapped between the ME list of this report and the male-specific epigenetically responsive genes to maternal periconceptual nutrition, while *MGMT* (**Fig. 1C**) and *CYP2E1* (**Fig. 2A**) matched between the female-specific list with our ME candidates.

MEs link to common human diseases. Further filtering of MEs—where 2 or more CpG sites met criteria within a 10 kb distance from each other—provided 401 loci (22.6% of the ME list), which associated with 140 genes (**Table S5**). DNA methylation at several of the MEs in which 3 or more CpG sites were found within 10 kb from each other has already been linked to common human disorders, including glioblastoma (*GSTM5*[11]), bladder cancer (*AQP11*[12]), breast cancer (*NAV1*[13]), colorectal cancer (*APC2*[14]), gastric cancer (*EBF3*[15]) and other cancers (*KCNK15*[16]), ileal Crohn disease (*S100A13*[17]), Parkinson disease (*CYP2E1*[18]) and bipolar disorder (*HCG9*[19]) (**Table S5**; **Fig 2A and B**).

MEs overlap with epigenetic discordance between monozygotic twins. Interindividual genetic variation can associate with epigenetic variation.[20,21] However, epigenetic variation in genetically identical monozygotic (MZ) twins is devoid of such bias. Therefore, we matched the ME loci with CpGs in which DNA methylation differences within at least 1 out of 6 MZ twin pairs exceeded 10% [MZ PBL DNA samples were obtained form the Danish twin registry (see ref. 4); for raw data see: www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32148]. Two hundred and forty-eight (14%) of the ME CpGs matched with the MZ twin list of CpGs in which at least 1 MZ pair showed methylation differences greater than 10% in PBL DNA (**Table S6**), while there was significantly fewer CpGs matching that criteria (2.6%;

Fischer exact p < 0.0001) in a control list of CpGs in which DNA methylation variation did not correlate between PBL and colonic mucosa (r < 0.5; p > 0.14). This result underscores that interindividual DNA methylation variation at a significant number of ME candidates can occur independently from genetic differences.

MEs overlap with genes in which DNA methylation correlates with gene expression. Direct correlation between MEs and gene expression at nearby genomic regions is of major functional significance. However, it should be noted that epigenetic changes can influence rather distant areas of the genome compared with where the changes take place. Therefore, any area of the genome behaving as an ME may bear potential physiologic importance. Nevertheless, we examined how the ME candidates identified in this study compare with genes whose expression correlated with DNA methylation variation in the colonic mucosa of control children. Since further biopsies from the discovery cohort of this study were not available, we examined DNA methylation and gene expression associations in the colonic mucosa of 5 other control patients (**Table S1B**).

There were 1181 CpG sites in which DNA methylation correlated (r > 0.871; p < 0.055) with the expression of 904 genes in the colonic mucosa of children, as determined by associating Infinium HumanMethylation450 v1.1 probe gene annotations to Affymetrix GeneChip PrimeView Human Gene Expression Array probe annotations (**Table S7**). One hundred and eighteen (11.6%) of the ME genes overlapped with this independent list. This overlap was significantly higher (Yates Chi square p < 0.0001) than expected by chance (2.8%). Additionally, the ME-associated, transcriptionally correlating genes had functional overrepresentation in biological processes involved in cell adhesion and development of anatomical structures in multicellular organisms (**Table S4B**). Furthermore, the transcriptionally relevant ME candidates maintained the significant association with biological function (Fatigo) and proteins (Interpro) at the level of homophilic cell adhesion and cadherin, respectively, similar to our observations in the list of MEs based only on methylation characteristics (**Table S4A and B**).

NetworkMiner (Babelomics4.3) revealed a functional link between 26 of the 118 transcriptionally relevant MEs (**Fig. S2**) that was significantly higher (p < 0.034) than in a randomly selected list of 2000 genes.

Importantly, 35 (25%) out of the 140 filtered (with 2 or more candidate CpG sites within 10 kb, see above) ME associated genes overlapped with the epigenetically linked gene expression in the colonic mucosa of children from the independent cohort. This association was significantly higher (Fischer exact p < 0.0001) than with the original list, indicating that the clustering of ME candidate CpGs predicts the transcriptional relevance of the methylation changes identified. *NAV1, EBF* and *KCNK15* (see above) still remained among these 35 genes, further implicating the role of MEs in human tumorigenesis.

Discussion

The term "metastable epiallele" was introduced by Emma Whitelaw and colleagues to describe mammalian alleles in which
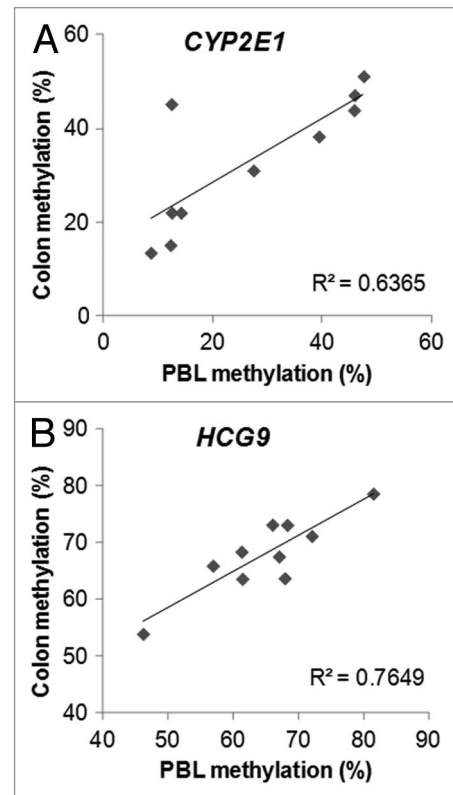
Figure 2. Examples for metastable epiallele (ME) candidates with 3 or more CpG sites meeting ME criteria in close vicinity (within 10 kb) of each other. DNA methylation from peripheral blood leukocyte (mesodermal origin) DNA, and colonic mucosa (colon, endodermal origin) showed significant intraindividual correlation, but > 10% interindividual methylation differences. (**A**) *CYP2E1*, where gene expression associated with DNA methylation in Parkinson disease brain. Epigenetic responsiveness to periconceptual maternal micronutrient supplementation was also observed in the cord blood of female newborns at this gene. (**B**) *HCG9*, where DNA methylation associated with bipolar disorder in three different [brain, white blood cells, sperms (germ line)] tissues (see text for references and details). n = 10. *CYP2E1*: r = 0.8, p = 0.005; *HCG9*: r = 0.87, p = 0.001.

variable expression associated with epigenetic differences instead of genetic heterogeneity. The murine *agouti viable yellow* (*A^vy*) is an outstanding example of such an allele.[22] Similarly behaving loci, even in imprinted genomic regions, have been observed in humans as well.[23]

The seminal work of Cooney and colleagues demonstrated that maternal micronutrient supplementation can shift DNA methylation distribution and the corresponding fur phenotype at the population level in *A^vy* mice, thereby implicating MEs in the nutritional (or environmental) origins of common human diseases.[24] Further studies confirmed the nutritionally driven establishment of interindividually variable, but intraindividually systematic DNA methylation to occur before gastrulation (i.e., in the periconceptual period)[25] and showed similar behavior at other mammalian MEs.[26] Post-gastrulation tissue-specific epigenetic modification can also occur at MEs, resulting in differing absolute levels of DNA methylation between the tissues of one individual, but the persistence of the similar magnitude of

interindividual methylation variation when the same tissues are compared between different organisms.[27] This could explain the behavior of DNA methylation at *C18orf22*, where methylation was lower in PBL DNA than in the colonic mucosa (**Fig. 1A**). In addition, the significant and similar interindividual variation between the select tissues persisted, implicating these loci as MEs.

The existence of human MEs was recently supported by the examination of PBL DNA from children in the Gambia.[1] In this geographic region, a bimodal annual variation of nutritional abundance (i.e., abundance and paucity of food within the same year) allowed for testing the effects of periconceptual maternal diet on offspring DNA methylation at a handful of putative MEs. A consecutive prospective study in the same genetically homogeneous population revealed a number of genomic loci in which infantile DNA methylation was modified as a result of maternal micronutrient supplementation in the periconceptual period.[10] Importantly, none of the 107 putative MEs from our original publication (see Table S2A from ref. 1) showed overlaps with the list of genes, which had DNA methylation associations with periconceptual nutrition from the cord blood of Gambian infants (see Supplemental Data set Table 2 and Gene list 1 and 3 from ref. 10). However, the candidate MEs identified in this work matched with 9.5% (**Fig. 2**) and 14.3% (**Fig. 1**) of these latter lists. Additionally, 8 (21.1%) genes (*AKAP12, CCDC102A, ITPKB, PANX2, PARD6G, SPNS2, SPTBN4* and *ZFYVE28*) from the former filtered putative human ME list (38 genes after filtering for interfering single nucleotide polymorphisms, copy number variations and segmental duplications: see Table S2C from ref. 1) had matches in our current compendium. Also, we observed significantly lower (2.6%; p = 0.028) association between the former putative MEs and our control list. This is a remarkable result, considering the different populations and tissues studied and the vastly dissimilar microarray methods employed between the two works.

The key feature of MEs is similar intraindividual methylation within all tissues of the body. Therefore, we specifically allowed either whole PBL or isolated PBMC DNA to be included on the arrays as DNA of mesodermal origin (see Materials and Methods) from the patients. Additionally, the diverse and clinically poorly defined (e.g., abdominal pain) proband inclusion allowed for the identification of common human ME candidates. With lack of whole genome sequencing we cannot rule out that interindividual genetic variation is the initiator of the epigenetic variation observed at many of the ME candidates identified. To address this issue, we filtered out CpGs from our candidate lists in which single nucleotide polymorphisms (SNPs) have been found to either overlap or to be near the loci. Nevertheless, trimodal or bimodal clustering of methylation values (such as at *PTN20B*; **Fig. 1C**) do raise the possibility for genetic (i.e., allelic) variation-stimulated DNA methylation differences. In addition, the homogeneous dispersion of interindividual systemic DNA methylation (i.e., significant correlation between DNA methylation from two tissues of different germ layer origin, leukocytes and colonic epithelium, within the same individual, such as at *C18orf22*, *CYPE1* and *HCG9*; **Figs. 1A and 2**) is more supportive of the ME status. Similarly, the overlaps with genes epigenetically sensitive to

periconceptual micronutrient supplementation in a genetically homogeneous population[10] (see above) argue for the non-genetic origins of DNA methylation differences at a number of our ME candidates. Furthermore, the significant overlap between inter-twin DNA methylation differences in MZ twin pairs and the ME list of this report indicates that a large number of the loci identified are true MEs. It should also be noted that the studied children were not healthy but had gastrointestinal complaints. Also, they provided a non-homogeneous population in respect to sex and age. They can only be considered as controls because anatomical abnormalities were not detected by colonoscopy or other examination of the intestines. Sex and age-dependent DNA methylation variation was excluded during the ME selection. However, it is possible that the clustering pattern of DNA methylation at select ME candidates may represent functional disease associations, such as subtypes of irritable bowel syndrome/recurrent abdominal pain, for example. In the meantime, no obvious discriminating phenotypic characteristics could be determined in association with methylation variation in the examples of **Figure 1 and 2**.

Within the highlighted MEs of this report (**Figs. 1 and 2**), *CYP2E1* in respect to Parkinson disease,[18] *HCG9* in bipolar disorder,[19] and *MGMT* in glioblastoma,[28] merit further consideration. The high monozygotic twin discordance rates[29] and lower prevalence of Parkinson disease (PD) in developing countries, such as Tanzania,[30] indicate the potential importance of environmentally-driven epigenetic changes, especially in developed countries, in the etiology of the disease. The increased cerebral expression of *CYP2E1* in association with decreased DNA methylation, its periconceptual epigenetic responsiveness,[10] and our findings suggesting it as an ME point toward *CYP2E1* as an outstanding candidate in respect to the developmental origins of PD. Similarly, the epidemiology of bipolar disorder (BPD) supports epigenetic factors playing a pathogenic role.[31] The association between BPD and *HCG9* methylation in 3 different tissues,[19] and our results indicating it as an ME, underscore the possible etiologic importance of *HCG9* in BPD. Furthermore, *MGMT* methylation within the tumor links the gene to earlier presentation with glioblastoma and predicts increased survival.[28] Epidemiologic observations show a tendency for increased incidence of gliomas in industrialized countries[32] pointing to the importance of environmental changes in this malignancy. The epigenetic responsiveness of *MGMT* to periconceptual intrauterine nutrition[10] and its potential ME nature outlined here indicate its role in the developmental origins of glioblastoma. Secondary to their ME nature, in addition to contributing to the pathology of human diseases, DNA methylation at *CYP2E1*, *HCG9* and *MGMT* may aid in the diagnostics (e.g., by determining relevant DNA methylation in the critically affected tissues based on PBL DNA methylation), prevention (by identifying and eliminating critical environmental influences responsible for the periconceptual DNA methylation changes), prognostics (*MGMT* methylation in PBL DNA may predict survival in glioblastoma, for instance) and even treatment of PD, BPD and glioblastoma in the future.

In order to determine the potential transcriptional relevance of the expanded ME compendium of this report, we examined the

correlation between DNA methylation and gene expression in the colonic mucosa of an independent group of control children. We could delineate 118 ME associated genes in which DNA methylation changes correlated with gene expression modification. Remarkably, the functional (homophilic cell adhesion) and protein (cadherin) based separation persisted between the methylation characteristics, and the further gene expression association based ME compendiums (**Table S4A and B**). Additional filtering of gene expression-correlated MEs defined 35 genes in association with 2 or more CpG sites within a 10 kb genomic region fulfilling the ME criteria. DNA methylation changes at a number of these genes have been associated with various forms of human disease, including cancers, such as asthma and acute myeloid leukemia (*ALOX12*), gastric cancer (*EBF3*), breast cancer (*NAV1*), colon cancer and acute lymphoid leukemia (*KCNK15*), Wilms tumor (protocadherin gene cluster; *PCDHAs*) and colorectal cancer (*TCERG1L*), suggesting a potential etiologic role for MEs in tumorigenesis and underscoring the possible developmental origins of these malignancies.

This is the first study to examine the potential transcriptional relevance of human MEs. Additionally, DNA methylation-associated common gene expression variation in histologically normal colonic mucosa of children was delineated. This latter list of genes will likely prove significant for future studies into pediatric gastrointestinal disorders. Based on the discussion above, the presented compendium of ME candidates may accelerate our understanding of the epigenetic developmental origins of multiple common human disorders.

Materials and Methods

Patients and PBL DNA isolation. Control patients (**Table S1A**) were recruited prior to endoscopy following informed consent through the institutional review board (IRB) approved tissue banks of the Charles University, Prague, Czech Republic (EK-1796/08) and the Pediatric Inflammatory Bowel Disease Consortium Registry at the Baylor College of Medicine (BCM; H-17654). The patients and data were included and described in a previous publication.[4] Further control patients examined by both DNA methylation and gene expression microarrays were recruited solely at BCM (**Table S1B**). Only patients with grossly and histologically normal mucosa at colonoscopy were designated as controls. For the peripheral blood leukocyte (PBL) DNA either Gentra Puregene (Qiagen) for whole blood DNA isolation was used according to manufacturer's recommendation on the samples from the Charles University, or the QuiAmp DNA mini kit (Qiagen) was utilized on isolated peripheral blood mononuclear cells (PBMCs) form the cohort of the Baylor College of Medicine. BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate was used in the latter case to isolate peripheral blood mononuclear cells (PBMCs) from PBL.

Tissues and nucleic acid processing for microarray. Colonic mucosa was obtained by pinch biopsies during endoscopy. Samples were flash frozen on dry ice and stored at -80°C until further processing. After thawing, the colonic mucosal biopsies were centrifuged at 14,000 rpm for 30 sec and resuspended in 500 μl RLT buffer (Qiagen) (with β-mercaptoethanol). Sterile 5 mm steel beads (Qiagen) and 500 μl sterile 0.1 mm glass beads (Scientific Industries, Inc.) were added for complete cellular lysis in a Qiagen TissueLyser (Qiagen), and run at 30 Hz for 5 min. Samples were centrifuged briefly, 350 μl of RTL and 200 μl of 100% ethanol were added to a 100 μl aliquot of the sample supernatant. This mixture was added to a DNA spin column, and DNA recovery protocols were followed as instructed in the QIAamp DNA Mini Kit (Qiagen) starting at step 5 of the Tissue Protocol. DNA was eluted from the column with 30 μl water and samples were diluted accordingly to a final concentration of 20 ng/μl. DNA samples were quantified using a Nanodrop spectrophotometer (Nyxor Biotech).

The PBL and colonic mucosal DNA samples following quality control with PicoGreen (http://probes.invitrogen.com/media/pis/mp07581.pdf) were processed by Infinium HumanMethylation450 BeadChip Kits (Illumina; www.illumina.com/products/methylation_450_beadchip_kits.ilmn) according to the manufacturer's recommendations through automated processes in the Core Laboratory for Translational Genomics of the Baylor College of Medicine. Arrays were imaged with BeadArray Reader using standard recommended Illumina scanner settings. GenomeStudio software version 2010.3.0.30128 was used to generate β values normalized to internal control probes. Internal controls determined the array processing to be of good quality. Only the 482,421 CpG probes on the array were used for subsequent analysis of the independent control samples (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=pvojjiqcqiuqori&acc=GSE42921). Bisulfite pyrosequencing validation of the arrays showed good correlation between DNA methylation levels and β values (ref. 4 and **Fig. S1**).

Colonic mucosal RNA was isolated by Qiangen-Qiazol miRNA Isolation Kit. cDNA amplification and labeling was performed with Ovation Pico WTA System V2 and Encore Biotin Module (NuGEN), respectively. Array hybridization was performed according to Affymetrix FS450_0002 Hybridization Protocol. The Affymetrix GeneChip® PrimeView™ Human Gene Expression Arrays were scanned with Affymetrix Genechip Scanner 7G. The R Bioconductor affy package rma function was used to compute expression. The CEL files and RMA normalized expression values can be downloaded from www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=pzyffweqggmsgnk&acc=GSE42911.

Metastable epiallele selection. MEs were selected by identifying CpG sites where the highest and the lowest methylation (based on β values) was in the same patients both from the peripheral blood leukocyte (PBL) and the colonic mucosal DNA. Only those CpG sites were selected where the average methylation difference (from PBL and colon) between individuals with the highest and the lowest methylation was ≥ 10% and ≤ 60% (to eliminate genetic bias arising from un-anticipated C > T polymorphisms), correlation (r) between PBL and colonic methylation was ≥ 0.64 or ≤ -0.64 (two tailed Pearson $p \leq 0.046$), and methylation was independent from age ($r \geq -0.6$ or ≤ 0.6; $p \geq 0.067$) and gender (male vs. female methylation two tailed unpaired T test $p > 0.05$). Finally, Infinium 450K probes which

contain SNPs at or near the target CpG site were removed based on the information here: http://ima.r-forge.r-project.org/. Gene associations were based on the Infinium HumanMethylation450 v1.1 manifest.

Methylation correlation with expression. Probe gene annotations in the Infinium HumanMethylation450 v1.1 manifest were used to identify associated probe gene annotations in the Affymetrix GeneChip PrimeView Human Gene Expression Array manifest based on matching gene annotations. HumanMethylation450 probes with a range of β values > 0.1 across the 5 samples were compared with PrimeView RMA normalized expression values using Pearson's correlation. Genes with β values and RMA normalized expression values correlated at r > 0.871, the $p < 0.055$ significance level for a sample size of 5, were used for subsequent analysis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Supplemental Materials

Supplemental materials may be found here: www.landesbioscience.com/journals/epigenetics/article/23438

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