

Whole-blood DNA Methylation Markers for Risk Stratification in Colorectal Cancer Screening: A Systematic Review

Janhavi R. Raut, Zhong Guan, Petra Schrotz-King and Hermann Brenner

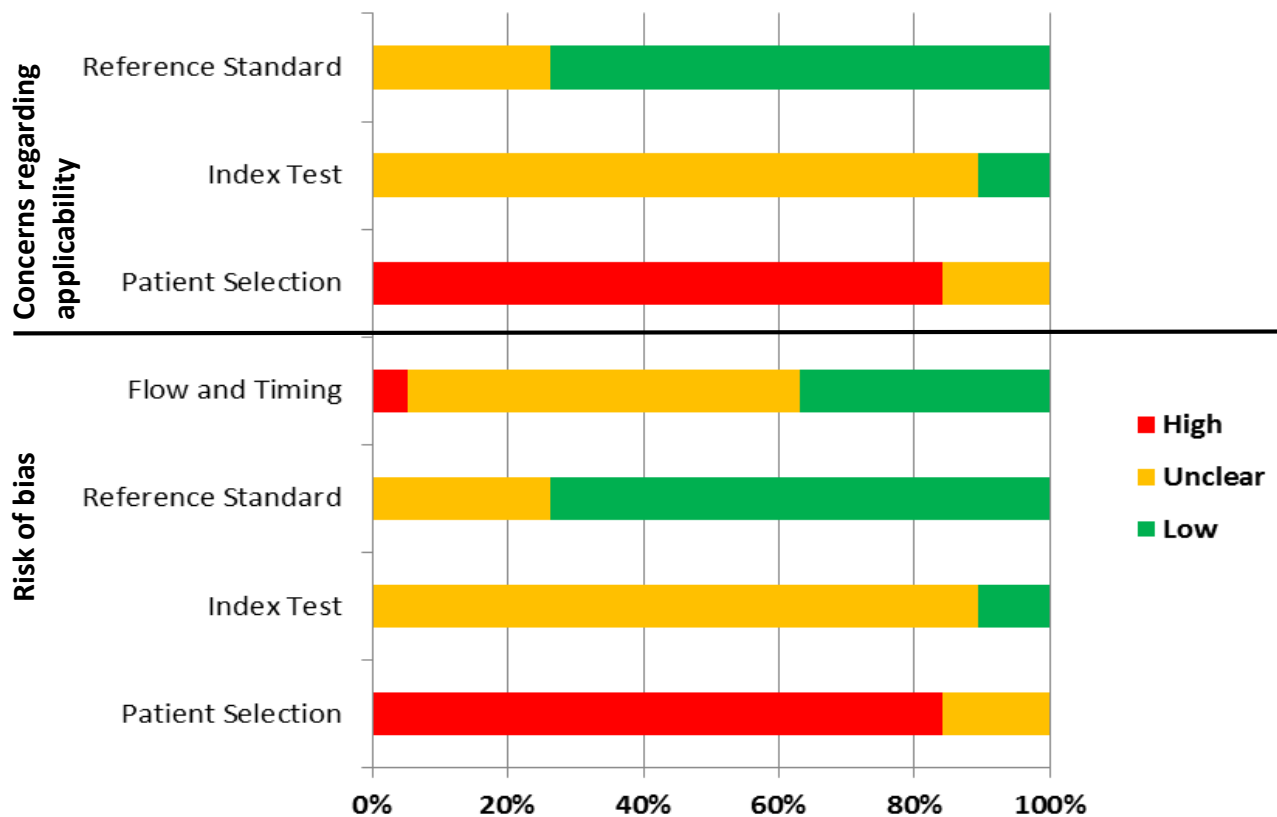


Figure S1. QUADAS Overview. Note: red bar, proportion with high risk; green bar, proportion with low risk; yellow bar, proportion with unclear risk.

	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Gao, 2018 [37]							
Zhang, 2017 [38]							
Liu, 2017 [39]							
Leclerc, 2017 [42]							
Heiss, 2017 [19]							
Alexander, 2017 [43]							
Luo, 2016 [40]							
Xiao, 2015 [41]							
Ravegnini, 2015 [32]							
Nüßgen, 2015 [36]							
Ho, 2015 [45]							
Gao, 2012 [27]							
Miroglio, 2010 [35]							
Kaaks, 2009 [28]							
Ally, 2009 [33]							
Ito, 2008 [34]							
Ashktorab, 2007 [44]							
Miotto, 2004 [31]							
Sabbioni, 2003 [30]							

 **High**
 **Unclear**
 **Low**

Figure S2. QUADAS-2 risk of bias assessment of reviewed studies [71].

Supplementary Table S1. Study characteristics of reviewed studies.

First author, Year [Ref. No.]	Study Design, Country	Study Group	Ascertainment of Cn by colonoscopy/ histopathology	No.	Mean Age (years)	DNAm assay	Reported gene/ LINE-1 region
Gao, 2018 [37]	Case-control China	CRC	Unclear	466	60.1	MS-HRM	<i>CA10</i> (-)
		Cn (non-gastrointestinal disease subjects)		507	56.7		<i>CRH</i> (-) <i>TMEM132D</i> (-) <i>CITED4</i> (-) <i>WT1</i> (-)
Liu, 2017 [39]	Case-control China	CRC	Unclear	428	59.4	MS-HRM	<i>APC</i> (-)
		Cn (Cancer-free subjects)		428	59.4		<i>CDH1</i> (-) <i>CDKN2A</i> (-) <i>DAPK1</i> (-) <i>IGF2</i> (-) <i>MGMT</i> (-) <i>MINT31</i> (-) <i>MLH1</i> (-) <i>NEUROG1</i> (-) <i>WIF1</i> (-)
Leclerc, 2017 [42]	Case-control USA	Cohort 1: CRC	Yes	40	59	Pyrosequencing	<i>PK4</i> (-)
		Cn (Polyps/ Polyp-free)		40	58		
		Cohort 2: CRC	Unclear	18	60		
		Cn (Cancer-free subjects)		29	60		

Supplementary Table S1. Cont.

First author, Year [Ref. No.]	Study Design, Country	Study Group	Ascertainment of Cn by colonoscopy/ histopathology	No.	Mean Age (years)	DNAm assay	Reported gene/ LINE-1 region	
Heiss, 2017 [19]	Case-control Germany	Screening Setting: CRC	Cn					
		(HPP/ normal colonic mucosa)	Yes	46	67		KIAA1549L (promoter, cg04036920	
		Clinical setting: CRC		46	67	HM 450K	& cg14472551)	
		Cn (from population registries)	Unclear	93	65	MassArray	BCL2 (body, cg12459502)	
							APC (promoter)	
							BRCA1 (promoter)	
							APBA1 (promoter)	
							PER1 (promoter)	
							PER3 (promoter)	
							SFRP4 (promoter)	
							SFRP5 (promoter)	
							TIMP3 (promoter)	
Alexander, 2017 [43]	Case-control USA	Adenoma (non-advanced)		38			TMEFF2 (promoter)	
		Cn (HPP/ normal colonic mucosa)	Yes	69	-	MS-PCR	WIF1 (promoter)	
							CDKN2A (promoter)	
							CYP27B1 (promoter)	
							ESR1 (promoter)	
							IGF2 (promoter)	
							MGMT (promoter)	
							MLH1 (promoter)	
							NGFR (promoter)	
							PER2 (promoter)	

Supplementary Table S1. *Cont.*

First author, Year [Ref. No.]	Study Design, Country	Study Group	Ascertainment of Cn by colonoscopy/ histopathology	No.	Mean Age (years)	DNAm assay	Reported gene/ LINE-1 region
Alexander, 2017 [43]	Case-control USA	Adenoma (non-advanced) Cn (HPP/ normal colonic mucosa)	Yes	38 69	-	MS-PCR	<i>SEPT9 (promoter)</i>
Zhang, 2017 [38]	Case-control China	Adenoma CRC Cn (healthy subjects)	Yes	20 50 20	52.6 69.4 49.2	qMS-PCR, Pyrosequencing	<i>CNRIP1 (promoter)</i>
Luo, 2016 [40]	Case-control China	CRC Cn (non-gastrointestinal disease subjects)	Unclear	421 506	59.5 56.6	MS-HRM	<i>IRF4 (-)</i> <i>FOXE-1 (-)</i> <i>AOX-1 (-)</i> <i>ADAMTS9 (-)</i> <i>RERG (-)</i> <i>RARB2 (-)</i>
Xiao, 2015 [41]	Case-control China	CRC Cn (healthy subjects)	Unclear	84 16	56 median 55.6 median	n-MS-PCR	<i>NDRG4 (promoter)</i>
Ravegnini, 2015 [72]	Case-control Italy	CRC Cn (normal colonic mucosa)	Yes	27 26	67.5 59.6	MS-PCR	<i>SEPT9 (promoter)</i>
Nüsgen, 2015 [36]	Case-control Lithuania	CRC Cn (healthy subjects)	Unclear	21 59	--	Pyrosequencing	<i>LINE-1 loci: L1C2</i> <i>L1C6</i> <i>L1C10</i> <i>L1C11</i> <i>L1C20</i> <i>L1X1</i> <i>L1X3</i>

Supplementary Table S1. *Cont.*

First author, Year [Ref. No.]	Study Design, Country	Study Group	Ascertainment of Cn by colonoscopy/ histopathology	No.	Mean Age (years)	DNAm assay	Reported gene/ LINE-1 region
Nüsgen, 2015 [36]	Case-control Lithuania	CRC Cn (healthy subjects)	Unclear	21 59	--	Pyrosequencing	<i>L1X4a</i> <i>L1X5b</i> <i>L1X6b</i> <i>L1X8</i>
Ho, 2015 [45]	Case-control Canada	Adenoma (TA/ VA/ TVA) Cn (normal colonic mucosa)	Yes	181 88	--	Sequenom EpiTYPER	<i>MTHFR</i> (28 CpG sites, TSS & Gene-coding area) <i>DNMT3B</i> (66 CpG sites, TSS & Gene-coding area including 31350991-31350995, 31351072, 31351122, 31351136, 31351144, 31351172-31351174, 31351216, 31351226-31351230 & 31351260– 31351263)
Gao, 2012 [27]	NCC Finland	CRC Cn (Cancer-free subjects)	Unclear	221 219	58 58	Illumina GoldenGate Methylation Cancer Panel I	<i>DSP_P440_R</i> <i>MLH3</i> <i>FLT4</i> <i>INSR</i> <i>GLA</i> <i>PTCH2</i> <i>GSTM2</i> <i>HCK</i> <i>PLAGL1</i> <i>PARP1</i>

Supplementary Table S1. *Cont.*

First author, Year [Ref. No.]	Study Design, Country	Study Group	Ascertainment of Cn by colonoscopy/ histopathology	No.	Mean Age (years)	DNAm assay	Reported gene/ LINE-1 region
Gao, 2012 [27]	NCC Finland	CRC Cn (Cancer-free subjects)	Unclear	221 219	58 58	Illumina GoldenGate Methylation Cancer Panel I	COL18A1
							KCNK4
							GJB2
							SEMA3F
							HBII
							SEMA3C
							MME_
							WNT1_
							SGCE
							PDE1B_
							HIC1
							PODXL
							IL18BP
							DKC1
Miroglio, 2010 [35]	Case-control France	CRC Cn (healthy subjects)	Unclear	26 19	64 40	Pyrosequencing	IGF2 (DMR0 between 2 nd & 3 rd exons)
							IGF2 (DMR2 between 8 th & 9 th exons)
							H19 DMR (4-kb upstream of the TSS)

Supplementary Table S1. *Cont.*

First author, Year [Ref. No.]	Study Design, Country	Study Group	Ascertainment of Cn by colonoscopy/ histopathology	No.	Mean Age (years)	DNAm assay	Reported gene/ LINE-1 region
Kaaks, 2009 [28]	NCC Sweden	CRC	Unclear	97	58	SOMA	<i>IGF2 (DMR in 2nd intron)</i>
		Cn (Alive and Cancer-free subjects)		190	58		
Ally, 2009 [33]	Case-control UK	Adenoma (≥1 adenoma; TA/ VA/ TVA/ SA)	Yes	30	65.5	Pyrosequencing	<i>ESR1(promoter & 1st exon)</i>
		CRC		27	68.4		
		Cn (normal colonic mucosa)		57	57.5		
Ito, 2008 [34]	NCC	Cohort 1: CRC	Unclear	225	66.1	Pyrosequencing	<i>IGF2 (DMR0 promoter proximal sequence CpG12-17)</i>
	UK	Cn		435	66.1		
	CS	Cohort 2: CRC		188	--		
	UK	Cn		94	--		
Ashktorab, 2007 [44]	Case-control USA	Adenomatous polyp/ HPP	Yes	19	60.5	MS-PCR	<i>APC (promoter)</i>
		Cn (Polyp-free)		29	63.5		
Miotto, 2004 [31]	Case-control Italy	CRC	Unclear	46	--	Semi n-MS-PCR	<i>CDH4 (promoter)</i>
		Cn (healthy subjects)		17			
Sabbioni, 2003 [30]	Case-control Italy	CRC	Unclear	29	-	MS-PCR	<i>TMEFF2</i>
		Cn (healthy subjects)		16			

Abbreviations: Ref., Reference; No., Number; NCC., Nested Case control (nested within a prospective cohort study); CRC, colorectal cancer; Cn, controls; DNAm, DNA methylation; HPP, hyperplastic polyp; TA, tubular adenomas; TVA, tubulovillous adenomas; VA, villous adenomas; SA, serrated adenomas; MS-PCR, Methylation-specific Polymerase Chain Reaction; q-MS-PCR, quantitative Methylation-specific Polymerase Chain Reaction; n-MS-PCR, nested Methylation-specific Polymerase Chain Reaction; MS-HRM, Methylation-sensitive High-resolution Melting; HM-450K., Human Methylation 450 k; SS, Screening Setting; CS, Clinical setting; DMR., Differentially Methylated Region; TSS, Transcriptional Start Site; SOMA, short oligonucleotide mass analysis.

Supplementary Table S2. DNA methylation difference between colorectal adenoma/ CRC cases and controls reported by P-value only.

Gene/ LINE-1 locus	First author, Year [Ref. No.]	Country	Study Group	No.	Age (years)	DNA methylation assay	p- value*
Hypomethylation							
<i>IGF2</i>	Miroglio, 2010 [35]	France	CRC Controls	26 19	64 40	Pyrosequencing	0.045
<i>PDK4</i>	Leclerc, 2017 [42]	USA	Cohort 1: CRC	40	59	Pyrosequencing	<0.01
			Controls	40	58		
			Cohort 2: CRC	18	60		<0.05
			Controls	29	60		
<i>SEPT9</i>	Ravegnini, 2015 [32]	Italy	CRC Controls	27 26	67.5 59.6	MS-PCR	0.0006
Hypermethylation							
<i>CNRIP1</i>	Zhang, 2017 [38]	China	Adenoma	20	52.6	q- MS-PCR, Pyrosequencing	0.00
			CRC	50	69.4		0.008
			Controls	20	49.2		
<i>ESR1</i>	Ally, 2009 [33]	UK	Adenoma	30	65.5	Pyrosequencing	> 0.05
			CRC	27	68.4		0.17
			Controls	57	57.5		
<i>L1X5b</i>	Nüsgen, 2015 [36]	Lithuania	CRC Controls	21 59	--	Pyrosequencing	0.04 ^a

^a p-value reported for females only. * p-value represents difference of DNA methylation between cases and controls. Abbreviations: Ref., Reference; No., Number; CRC, colorectal cancer; MS-PCR, Methylation-specific Polymerase Chain Reaction; q- MS-PCR, quantitative Methylation-specific Polymerase Chain Reaction.

Supplementary Table S3. Methylation of genes with no significant association to colorectal adenoma/ CRC risk.

Gene	First author, Year [Ref. No.]	Country	Study Group	No.	Mean Age (years)	DNAm assay	OR value (95%CI)	<i>p</i> - value
Hypomethylation								
<i>APC</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	1.0 (0.4–2.5) ^{a, c}	0.97
<i>IGF2</i>	Ito, 2008 [34]	UK	Cohort 1: CRC Controls	225 435	66.1 66.1	PS	1.09 (0.65–1.82) ^{b, d}	0.74
<i>SFRP4</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	1.1 (0.3–3.8) ^a	0.89
<i>TIMP3</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	1.2 (0.4–3.0) ^a	0.78
<i>WIF1</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	1.3 (0.5–3.2) ^a	0.56
<i>TMEFF2</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	1.5 (0.6–3.4) ^a	0.36
<i>BRCA1</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	1.5 (0.6–3.9) ^a	0.36
<i>IGF2</i>	Ito, 2008 [34]	UK	Cohort 2: CRC Controls	192 96	65.9 43.2	PS	1.63 (0.06–42.5) ^{b, d}	0.77
<i>SFRP5</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	3.7 (0.8–17.1) ^a	0.09
Hypermethylation								
<i>CRH</i>	Gao, 2018 [37]	China	CRC Controls	466 507	60.1 56.7	MS-HRM	0.54 (0.25–1.19) ^e	0.13
<i>PER2</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.6 (0.1–3.0) ^f	0.56
<i>ESR1</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.6 (0.3–1.5) ^g	0.27
<i>CDH1</i>	Liu, 2017 [39]	China	CRC Controls	428 428	59.4 59.4	MS-HRM	0.65 (0.36–1.19) ^h	0.16

Supplementary Table S3. *Cont.*

Gene	First author, Year [Ref. No.]	Country	Study Group	No.	Mean Age (years)	DNAm assay	OR value (95%CI)	<i>p</i> - value
<i>MLH1</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.7 (0.2–3.1) ⁱ	0.67
<i>DNMT3b</i> (GCA, 31351216)	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	0.72 (0.31–1.65) ^{j, m}	0.17
<i>CYP27B1</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.8 (0.2–3.0) ⁱ	0.72
<i>IGF2</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.8 (0.3–2.0) ^k	0.57
<i>CDKN2A</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.8 (0.3–1.9) ^g	0.54
<i>NGFR</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.9 (0.3–2.2) ^c	0.75
<i>SEPT9</i>	Alexander, 2017 [43]	USA	Adenoma Controls	38 69	--	MS-PCR	0.9 (0.4–2.1) ^c	0.81
<i>RARB2</i>	Luo, 2016 [40]	China	CRC Controls	421 506	59.5 56.6	MS-HRM	0.96 (0.70–1.30) ^l	0.78
<i>IGFII CpG2</i>	Kaaks, 2009 [28]	Sweden	CRC Controls	97 190	58 58	SOMA	1.00 (0.46–2.19) ^m	0.58 ⁿ
<i>IGFII CpG1</i>	Kaaks, 2009 [28]	Sweden	CRC Controls	97 190	58 58	SOMA	1.02 (0.49–2.16) ^m	0.96 ⁿ
<i>DNMT3b</i> TSS	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.11 (0.49–2.51) ^{j, m}	0.71
<i>MTHFR</i> GCA	Ho, 2015 [45]	Canada	Adenoma Controls	181 88	--	Sequenom EpiTYPER	1.11 (0.51–2.44) ^{j, m}	0.96
<i>DNMT3b</i> (GCA, 31351122)	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.14 (0.49–2.42) ^{j, m}	0.81
<i>MTHFR</i> TSS	Ho, 2015 [45]	Canada	Adenoma Controls	181 88	--	Sequenom EpiTYPER	1.17 (0.54–2.51) ^{j, m}	0.89

Supplementary Table S3. *Cont.*

Gene	First author, Year [Ref. No.]	Country	Study Group	No.	Mean Age (years)	DNAm assay	OR value (95%CI)	<i>p</i> - value
<i>DNMT3b</i> Average	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.25 (0.54–2.86) ^{j, m}	0.49
<i>DNMT3b</i> (GCA, 31351172- 31351174)	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.32 (0.59–2.95) ^{j, m}	0.57
<i>FOXE-1</i>	Luo, 2016 [40]	China	CRC Controls	421 506	59.5 56.6	MS-HRM	1.35 (0.99–1.85) ^l	0.06
<i>MTHFR</i> Average	Ho, 2015 [45]	Canada	Adenoma Controls	181 88	--	Sequenom EpiTYPER	1.45 (0.68–3.12) ^{j, m}	0.47
<i>TMEM132D</i>	Gao, 2018 [37]	China	CRC Controls	466 507	60.1 56.7	MS-HRM	1.52 (0.99–2.34) ^e	0.06
<i>DNMT3b</i> (GCA, 31351072)	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.61 (0.67–3.88) ^{j, m}	0.57
<i>DNMT3b</i> (GCA, 31350991 - 31350995)	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.65 (0.77–3.53) ^{j, m}	0.11
<i>MLH1</i>	Liu, 2017 [39]	China	CRC Controls	428 428	59.4 59.4	MS-HRM	1.72 (0.68–4.34) ^h	0.25
<i>APC</i>	Liu, 2017 [39]	China	CRC Controls	428 428	59.4 59.4	MS-HRM	1.82 (0.74–4.49) ^h	0.19
<i>DNMT3b</i> (GCA, 31351226 - 31351230)	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.86 (0.83–4.15) ^{j, m}	0.48
<i>CDKN2A</i>	Liu, 2017 [39]	China	CRC Controls	428 428	59.4 59.4	MS-HRM	1.99 (0.56–7.04) ^h	0.29
<i>DNMT3b</i> (GCA, 31351144)	Ho, 2015 [45]	Canada	Adenoma Controls	87 172	--	Sequenom EpiTYPER	1.99 (0.88–4.50) ^{j, m}	0.37
<i>APC</i>	Ashktorab, 2007 [44]	USA	AP/HPP Controls	19 29	60.5 63.5	MS-PCR	3.6 (0.6–22.0)	0.15

^a Odds of adenoma development given no methylation detected in the candidate gene. ^b Odds of CRC development given loss of methylation detected in the candidate gene. OR: ^c Model adjusted for vitamin D and multivitamin use; ^d Model adjusted for BMI, number of packet smoked per year and family history of cancer; ^e Model adjusted for BMI, age, fruit, coarse grains, fruit can, pork intestines, fried food, garlic, and braised fish in brown sauce; ^f Model adjusted for vitamin D and multivitamin use, physical activity, and age group; ^g Model adjusted for vitamin D use, physical activity, and age; ^h Model adjusted for age, gender, BMI, occupational physical activity, smoking, and consumption of coarse grains, fish stewed with brown sauce, fried food, leftovers and pork; ⁱ Model adjusted for vitamin C and D use, physical activity, and age; ^j Model adjusted for sex and age; ^k Model adjusted for vitamin C and D use and physical activity; ^l Model adjusted for age, BMI, occupation and family history of cancer. ^m OR for Quartile 4 vs 1. ⁿ p-value reported for trend across quartiles of methylation level. Abbreviations: Ref., Reference; No., Number; DNAm, DNA methylation; CRC, colorectal cancer; AP, adenomatous polyp; HPP, hyperplastic polyp; MS-PCR, Methylation-specific Polymerase Chain Reaction; MS- HRM, Methylation-sensitive High-resolution Melting; PS, Pyrosequencing; SOMA, short oligonucleotide mass analysis.

