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Review

The CpG island methylator phenotype in colorectal cancer: Progress and problems

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ABSTRACT

In recent years, attention has focused on the biology and potential clinical importance of the CpG island methylator phenotype (CIMP) in colorectal cancer (CRC). While it is generally well accepted that etiologically and clinically distinct subgroups exist in this disease, a precise definition of CIMP remains to be established. Here, we summarize existing literature that documents the prevalence of CIMP in CRC, with particular attention to the various methods and definitions used to classify a tumor as CIMP positive. Through a systematic review on both case-series and population based studies, we examined only original research articles reporting on sporadic CRC and/or adenomas in unselected cases. Forty-eight papers published between January 1999 and August 2011 met the inclusion criteria. We describe the use of multiple gene panels, marker threshold values, and laboratory techniques which results in a wide range in the prevalence of CIMP. Because there is no universal standard or consensus on quantifying the phenotype, establishing its true prevalence is a challenge. This bottleneck is becoming increasingly evident as molecular pathological epidemiology continues to offer possibilities for clear answers regarding environmental risk factors and disease trends. For the first time, large, unselected series of cases are available for analysis, but comparing populations and pooling data will remain a challenge unless a universal definition of CIMP and a consensus on analysis can be reached, and the primary cause of CIMP identified.

Contents

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1. Introduction

Altered epigenetic regulation of gene expression in cancer is organized at multiple levels and involves DNA methylation, histone modifications, chromatin remodeling and non-coding RNAs [1]. Such modifications are of interest to cancer prevention, detection and management strategies because they can be present in pre-cancerous 'normal' tissue, thereby modifying cancer risk [2–4], and are associated with both cancer initiation and progression [3,5,6]. Furthermore, it is becoming clear that epigenetic aberrations are promising molecular markers for early detection and markers of prognosis and response to therapy [7–9].

The most studied epigenetic alteration is DNA hypermethylation of promoter-associated CpG islands of tumor suppressor and DNA repair genes, which is now recognized as a common feature of human neoplasia as it leads to transcriptional silencing of the gene [10]. Widespread CpG island promoter methylation, also referred to as the CpG island methylator phenotype (CIMP) [10–13], has been reported in several tumor types, including gastric [14–19], lung [20,21], liver [22], ovarian [23], glioblastomas [24], endometrial [25,26], breast [27] and leukemias [28,29]. However, the term was first coined [30] and the phenotype has been most studied in colorectal cancer (CRC).

CRC tumors characterized by CIMP are thought to arise via the serrated neoplasia pathway [31], and have distinctly different histology when compared to tumors derived from traditional adenoma-carcinoma pathway [32–35]. An early event in CIMP tumors appears to be a mutation in the *BRAF* proto-oncogene, which inhibits normal apoptosis of colonic epithelial cells [34]. In addition, most CIMP CRCs are characterized by promoter CpG island methylation of the mismatch repair gene, *MLH1*, resulting in its transcriptional inactivation. Loss of *MLH1* is thought to cause microsatellite instability (MSI), a form of genetic instability characterized by length alterations within simple repeated microsatellite sequences of DNA [36,37]. Once *MLH1* is inactivated, the rate of progression to malignant transformation is rapid [34]. Clinically, there is evidence to suggest that CIMP is associated with prognosis [38,39] and it is also being investigated as a predictive marker for response to chemotherapy treatment [40–42]. Descriptively, tumors of the serrated neoplasia pathway are associated with older age, female sex, and tumors of the proximal colon [11,43–47]. Furthermore, CIMP has been investigated in association with a number of environmental risk factors as was recently reviewed by Curtin et al. [48].

Although it has been more than a decade since CIMP was first identified in CRC, the path to accepting these tumors as an etiologically and clinically distinct group of the disease has not been without controversy (Fig. 1), and to date, the cause of CIMP remains unknown. Moreover, there is no gold standard with respect to gene panels, marker thresholds or techniques for detection of the altered DNA methylation used to define this phenotype. Here, we systematically review the literature to provide a synopsis of current knowledge on CIMP in CRC research, shedding light on the need for universal consensus guidelines.

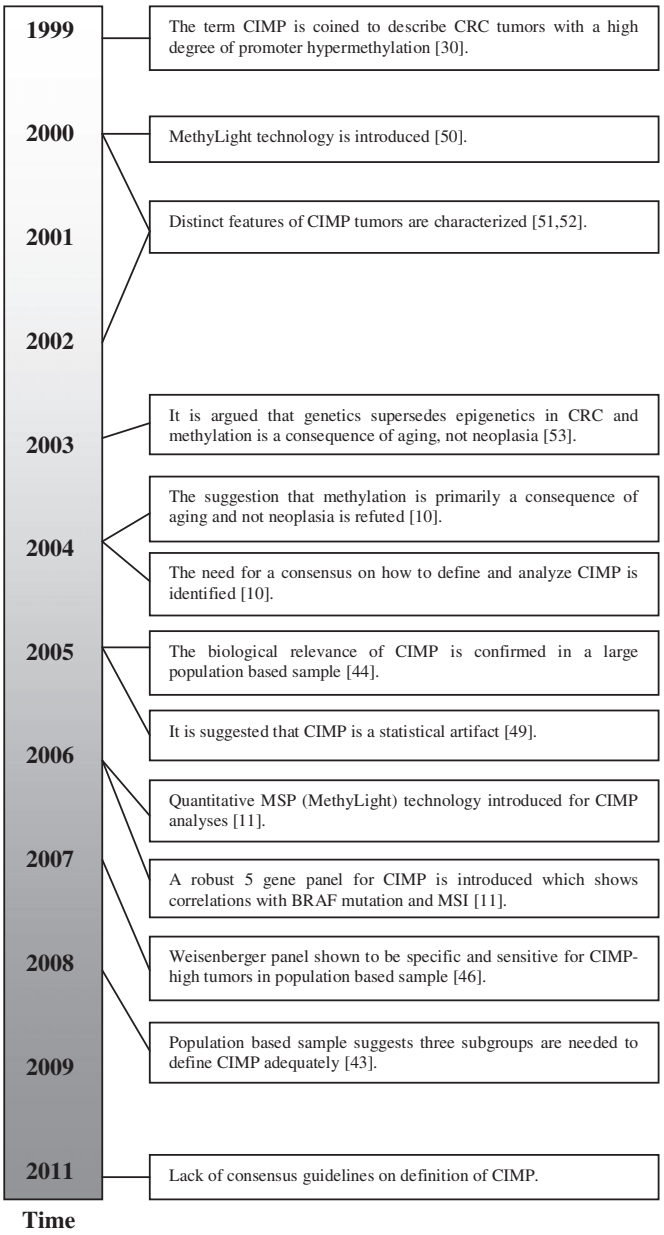

Fig. 1. A timeline of the major developments surrounding CIMP over the past decade [10,11,30,43,44,46,49,50–53].

2. Methods

2.1. Criteria for inclusion

Articles eligible for this review were studies that reported on the prevalence of CIMP in sporadic CRC and/or colorectal adenomas. Furthermore, only original articles (i.e. not reviews or editorials) that consisted of unselected cases were considered.

2.2. Search strategies

A systematic review was performed for all English language articles until June 2011 in three databases: MEDLINE, PUBMED, and EMBASE. The keywords used were combined uniformly and extensively in each database and included: adenocarcinoma; cancer; carcinoma; cimp; colon; colonic; rectum; rectal; colorectal; cpg island methylation phenotype; cpg island methylator phenotype; neoplasia; neoplasm. The articles identified by the search were registered in an Endnote database without duplicates. Articles were first selected or excluded based on title. Then, abstracts and full text of articles were reviewed for the inclusion criteria. This scheme is outlined in Fig. 2.

2.3. Assessment of validity and data extraction

Two authors (LH and CK) independently screened all retrieved reports and selected those that were potentially valid. The discrepancies of validity assessment were resolved by discussion with MvE. Information was sought for the following four criteria: method used to detect CIMP, gene panel used to define CIMP, threshold for CIMP positivity, and CIMP prevalence. An electronic, standardized registration form was used for data extraction from the selected articles and

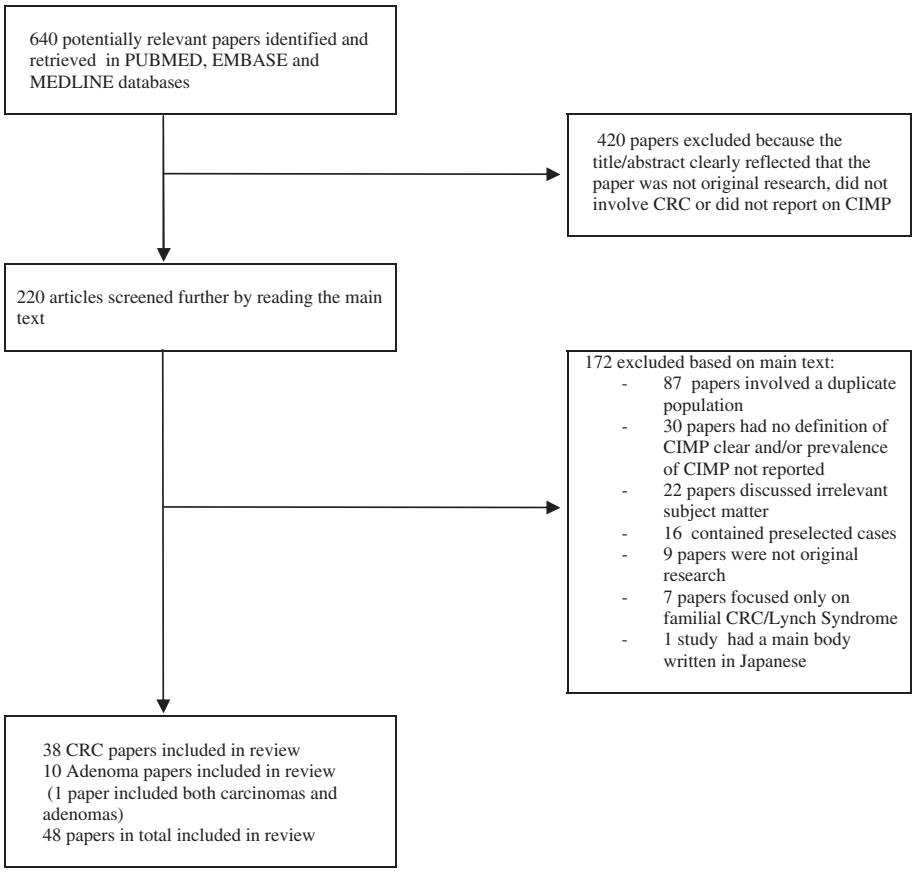

Fig. 2. Flow diagram of study selection process.

included information on research group, country, characteristics of population, study size, study design, methods, CIMP panel used, threshold for CIMP positivity, CIMP prevalence.

3. Results

3.1. Search results

Searching the PUBMED, EMBASE and MEDLINE databases initially yielded a total of 754 citations. After excluding duplicates, 640 articles remained, and 420 were discarded on the basis of a title clearly not reflecting the inclusion criteria (not an original article, not involving sporadic CRC, not reporting on CIMP). Of the remaining 220 articles, 172 were further discarded after reading the main text (87 papers included a duplicate study population; 30 papers contained no clear definition of CIMP and/or the prevalence of CIMP was not reported; 22 papers discussed methylation, but not CIMP; 16 papers described pre-selected cases (i.e. tumors characterized by MSI or BRAF mutation); 9 papers were not original research; 7 papers described familial CRC or Lynch Syndrome cases; and 1 paper had a main body written in Japanese). Thus, 48 publications from January 1999–August 2011 were ultimately included in this review. These included 38 papers reporting on sporadic CRC cases, and 10 papers reporting on colorectal adenomas, and 1 paper described both adenomas and carcinomas.

3.2. Description of studies

The characteristics of included studies are summarized in Table 1. The 38 publications on sporadic CRC came from 15 different countries, and included 26 case series, 3 population based series, 2 articles reporting case–cohort data (one study population; colon and rectum were reported separately [54,55]), and 6 articles reporting prospective cohort data (from 5 populations; one population reports on 2 different gene panels [13,46]). One paper also reported population based case-series and prospective cohort data in the same paper [38]. With respect to studies involving case series, 5 different laboratory techniques were used to quantify CIMP, 12 different gene panels were used to define CIMP, and marker thresholds varied depending on, and also within, gene panels. With respect to population based studies, 3 different methods and 4 different gene panels were utilized to define CIMP. The 11 publications on colorectal adenomas spanned 4 countries and were all case series. Two different laboratory techniques and 5 different gene panels were used to define CIMP.

Many different combinations of gene panel/marker thresholds/laboratory methods were used to quantify CIMP, and furthermore, it is difficult to give a range of the prevalence of CIMP, because the variation in observed prevalence is partly dependent on characteristics known to be associated with CIMP, such as location (i.e. studies only reporting only on colon tumors will likely have a higher prevalence of CIMP than studies reporting on both colon and rectum tumors). In general, we observed no clear patterns indicating that specific gene panels and/or laboratory technique gave consistently a higher or lower CIMP prevalence.

3.3. Gene panels and marker thresholds

The so-called 'classic panel', which includes *MINT1, MINT2, MINT31, CDKN2A (p16)* and *MLH1*, was identified by a PCR-based analysis of SmaI digestion sites and has been used since then [10]. In 2006, a robust five gene panel was introduced by Weisenberger et al. [11], which includes the genes *CACNA1G, IGF2, NEUROG1, RUNX3* and *SOCS1*. This panel was identified by unsupervised

Table 1
Summary of studies of CIMP detection and status.

Study	Study characteristics		Tumor	Gene panel[a]	Assessment of CIMP		% CIMP-H[b] subsite		
	Country	N			Method	Marker threshold to assign CIMP-H[b]			
Sporadic CRC							*Proximal*	*Distal*[g]	*Overall*
Case series									
An et al., 2010 [62]	Japan	94	Colorectal	Classic panel[c]	Bisulfite pyrosequencing	≥2/5 methylated	43	17	28
Chan et al., 2005 [63]	Egypt	93	Colorectal	Classic panel	Methylation specific PCR	≥2/5 methylated	–	–	22
	Turkey	95			(MSP)		–	–	37
	Jordan	59					–	–	35
Frazier et al., 2003 [64]	USA	47	Colorectal	Classic panel	MSP	≥3/5 methylated	–[h]	–	45
Lee et al., 2008 [65]	South Korea	134	Colorectal	Classic panel	MSP	≥2/5 methylated	44	23	31
Suehiro et al., 2008 [66]	USA	208	Colorectal	Classic panel	MSP	≥3/5 methylated	–	–	10
	Hong Kong						–	–	
O'Brien et al. 2006 [67]	USA	10 (SC)[f]	Colorectal	Classic panel	MSP	≥2/5 methylated	–	–	90
		59 (TCA)					–	–	39
Lee et al., 2008 [68]	South Korea	130	Colorectal	Classic panel	MSP	≥2/5 methylated	–	–	23
				Weisenberger panel[d]		≥3/5 methylated	67	33	19
						≥2/5 methylated	–	–	23
						≥3/5 methylated	64	36	17
Karpinski et al., 2010 [69]	Poland	186	Colorectal	Weisenberger panel	MSP	≥3/5 methylated	–	–	25
Ang et al., 2010 [70]	Australia	91	Colorectal	Weisenberger panel	MethyLight (PMR = 4)[e]	≥3/5 methylated	–	–	18
Greco et al., 2010 [71]	Australia	55	Colorectal	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	–	–	26
Iacopetta et al., 2007 [72]	Australia	205	Colon	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	85	15	17
Kawakami et al., 2008 [73]	Japan	150	Colorectal	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	–	–	9
Sanchez et al., 2009 [74]	USA	391	Colorectal	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	89	11	21
Weisenberger et al. 2006 [11]	Australia	187	Colorectal	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	67	33	18
Cheng et al. 2008 [75]	USA	161	Colon	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	–	–	20
Hinoue et al. 2011 [59]	Netherlands	125	Colorectal	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	86	15	22
Kim et al., 2009 [76]	South Korea	320	Colorectal	Weisenberger panel + *CDKN2A (p16), CRABP1, MLH1*	MethyLight (PMR = 4)	≥5/8 methylated	76	–	12
Arain et al. 2010 [77]	USA	167	Colon	*MINT1, MINT2, MINT31, P16INK4, MGMT, MLH1*	Real Time PCR	≥3/6 methylated	58	42	38
Goel et al., 2007 [60]	Canada, Germany	126	Colon	*MINT1, MINT2, MINT31, p16INK4, MGMT, MLH1*	MSP	≥3/6 methylated	–	–	31
	USA								
	Japan								
Cai et al., 2008 [78]	China	69	Colon	*p14ARF, MLH1, p16INK4, MGMT, MINT1*	MSP	≥3/5 methylated	–	–	18
Deng et al., 2008 [79]	USA	74	Colorectal	*MLH1, p16ink4A, HIC1, RASSF2, MINT1, MINT31 + SFRP1, SFRP2, SFRP4, SFRP5 + SLC5A8, TAC1, SST MGMT*	MSP	≥3/6 markers	35	65	24
						≥9/14 markers	38	62	31
						≥12/14 markers	100	0	9
Kakar et al., 2008 [80]	USA	83	Colorectal	*MLH1, p16, HIC1, RASSF2, ID4, MINT1, MINT31*	MSP	≥3/7 methylated	–	–	34
Kambara et al., 2004 [81]	Australia	145	Colorectal	*MINT1, MINT2, MINT12, and MINT31*	COBRA	≥3/4 methylated	–	–	25
Kim et al. [82]	South Korea	285	Colorectal	*MLH1, MINT1, MINT2, MINT31, p16INK4a, p14ARF, CACNA1G*	Bisulfite Pyrosequencing	≥2/7 methylated	–	–	36
Sugai et al., 2006 [83]	Japan	119	Colorectal	*MINT1, MINT2, MINT31, p14, p16, MGMT, MLH1, RASSF-1A*	MSP	≥3/8 methylated	51	25	32
Toyota et al., 1999 [30]	USA	41		*MLH1, MINT1, MINT2, MINT12, MINT31 and p16*	MSP	≥3/6 methylated	82	37	51
Ahn et al. 2011 [61]	South Korea	161	Colon	*MINT1, MINT2, MINT31, hMLH1, p16, p14,* and *WNT5A*	Bisulfide pyrosequencing	≥3/7 methylated	–	–	18
Population based series									
Samowitz et al., 2005 [44]	USA	864	Colon	Classic panel	MSP	≥2/5 methylated	68	32	30

Barault et al., 2008 [43]	France	582	Colon	Classic panel	MSP	≥4/5 methylated	81	19	17
Jover et al., 2010 [84]	Spain	320	Colorectal	Weisenberger panel	Bisulfite pyrosequencing	≥3/5 methylated	–	–	30
Dahlin et al., 2010 [38]	Sweden	414	Colorectal	Weisenberger panel + *CDKN2A (p16), CRABP1, MLH1*	MethyLight (PMR = 4)	≥6/8 methylated	76	24	11
Case control									
Samowitz et al., 2006 [54]	USA	1143 cases	Colon	Classic panel	MSP	≥2/5 methylated	74	23	29
Slattery et al., 2010 [55]	USA	750 cases	Rectum	Classic panel	MSP	≥2/5 methylated	–	–	11
Prospective cohort									
de Vogel et al., 2008 [85]	Netherlands	120,852 (cases = 734)	Colorectal	Weisenberger panel	MSP	≥3/5 methylated	–	–	27
English et al., 2008 [86]	Australia	41,328 (cases = 717)	Colorectal	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	84	15	14
Limsui et al., 2010 [87]	USA	37, 399 (cases = 555) women only	Colorectal	Weisenberger panel	MethyLight (PMR = 10)	≥3/5 methylated	–	–	31
Ogino et al., 2006 [13]	USA	173,229 (cases = 460)	Colorectal	*CACNA1G, CDKN2A (p16), CRABP1, MLH1, NEUROG1*	MethyLight (PMR = 4)	≥4/5 methylated	–	–	17
Ogino et al., 2007 [46]	USA	173,229 (cases = 920)	Colorectal	Weisenberger panel + *CDKN2A (p16), CRABP1, MLH1*	MethyLight (PMR = 4)	≥6/8 methylated	–	–	15
Dahlin et al., 2010 [38]	Sweden	166,414 (cases = 190)	Colorectal	Weisenberger panel + *CDKN2A (p16), CRABP1, MLH1*	MethyLight (PMR = 4)	≥6/8 methylated	78	22	14
Adenomas[f]									
Case series									
Chan et al., 2002 [88]	USA	102 (HP)	Colorectal	Classic Panel	MSP	≥2/5 methylated	75	19	43
		8 (SA)					–	–	75
		19 (TA)					–	–	32
O'Brien et al., 2004 [89]	USA	79 (HP)	Colorectal	Classic Panel	MSP	≥2/5 methylated	80	23	51
Yang et al., 2004 [90]	USA	79 (HP)	Colorectal	Classic Panel	MSP	≥2/5 methylated	–	–	52
		25 (SA)					–	–	80
Hiraoka et al., 2006 [91]	Japan	205	Colorectal	Classic Panel	MSP	≥2/5 methylated	57	43	22
O'Brien et al., 2006 [67]	USA	14 (GCSP)	Colorectal	Classic Panel	MSP	≥2/5 methylated	–	–	14
		38 (MVSP)					–	–	47
		29 (SPAP)					–	–	76
		29 (SA)					–	–	79
		30 (sTA)					–	–	10
		27 (ITA)					–	–	44
Park et al., 2003 [92]	USA	22 (SSA)	Colorectal	Classic Panel	MSP	≥2/5 methylated	70	64	68
		34 (TA)							18
Vaughn et al., 2010 [93]	USA	52 (HP)	Proximal Colon	Weisenberger Panel	MethyLight (PMR = 10)	≥3/5 methylated	48	4	29
Velho et al., 2008 [94]	Portugal	17 (HP)	Colorectal	Weisenberger Panel	MSP	≥3/5 methylated	–	—	25
Rashid et al., 2001 [95]	USA	50	Colorectal	*p16, MINT2, MINT31*	MSP	≥2/3 methylated	23	29	25
Kim et al., 2005 [96]	South Korea	40	Colorectal	*APC, THBS1, MGMT, MLH1, GSTP1*	MSP	≥2/5 methylated	42	58	30
Kim et al., 2008 [97]	USA	48 (HP)	Colorectal	*MLH1, p16, HIC1, RASSF2, MGMT, MINT1,MINT31*	MSP	≥3/7 methylated	67	8	33
		32 (SSA)					50	36	44
		30 (SA)					75	32	43
		32 (TA)					25	31	28

[a] Gene names are reported as they were in the original study.
[b] CIMP-H refers to either CIMP or in the instance that a study reported three CIMP categories, CIMP-high.
[c] Classic panel includes the genes: *MINT1, MINT2, MINT31, CDKN2A(p16)* and *hMLH1.*
[d] Weisenberger panel includes the genes: *CACA1G, IGF2, NEUROG1, RUNX3 and SOCS1.*
[e] PMR = percentage of methylated reference.
[f] HP (hyperplastic polyps), SA (serrated adenoma), TA (traditional adenoma), GCSP (goblet cell serrated polyp), MVSP (microvesicular serrated polyp), SPAP (serrated polyp with abnormal proliferation), sTA (small traditional adenoma), lTA (large traditional adenoma), SC (serrated carcinomas), TCA (traditional carcinomas).
[g] Some studies only reported distal colon, whereas others reported distal location (distal colon + rectum).
[h] Data not reported according to CIMP status.

hierarchical clustering analysis of 92 MethyLight-analyzed cancer-specific CpG sites of 295 CRCs and independently confirmed the existence of CIMP as well as showed the association between CIMP and *BRAF* mutation. It has now been shown that *MINT1, MINT2,* and *MINT31* are not specific for *BRAF*-mutated CIMP tumors [11]. However, studies validating the Weisenberger markers [46,56] emphasized that such findings do not indicate that these *MINT* markers or other CpG islands are inappropriate for assessment of CIMP in CRC; there is a possibility that a difference in primer designs and PCR conditions may substantially change sensitivity and specificity of a particular marker for the detection of CIMP [46]. Most recently, Ogino et al. proposed that a panel of (at least) four markers including *RUNX3*, *CACNA1G*, *IGF2*, and *MLH1* should constitute a sensitive and specific CIMP panel for the purpose of research and clinical use [46], but it is unknown how many studies have adapted this advice. At present, no set criteria for defining an ideal panel of CIMP markers exists, and one may argue which criteria are most important to consider; i.e. strength of association with BRAF mutation, proximal location in the tumor vs. tumor specific methylation. Ultimately, genome-wide studies of methylation and hierarchical cluster analysis of the data may eventually reveal a distinct subgroup of CRC with very frequent methylation of functionally important tumor suppressor genes, from which a small panel could then be chosen.

Furthermore, there is debate whether CIMP should be distinguished as two categories ('CIMP' and 'non-CIMP') [11,30], three categories (either 'CIMP-high, CIMP low, CIMP-0' [57] or 'CIMP1, CIMP2, CIMP-negative [58]), or most recently, four categories (CIMP-high, CIMP-low and two clusters of non-CIMP depending on the frequency of *TP53* mutation) [59]. This stems from the observation that some tumors demonstrate an intermediate amount of aberrant DNA or a cluster with differentially methylated genes than the classical CIMP cluster. Recently, Kaneda et al. [3] reported that a two panel method utilizing two different sets of CIMP-related markers is required to properly classify CRC into one of three DNA methylation epigenotypes: high, intermediate and low.

In practice, two prospective cohort studies report similar prevalence using an 8 gene panel to distinguish the three categories proposed by Ogino et al. (the five genes in the Weisenberger panel plus *CDKN2A (p16), CRABP, and MLH1)* [38,46]. However, it was also reported that differences between CIMP-low (1/8 to 5/8 methylated promoters) and non-CIMP (0/8 methylated promoters) were not large [46].

3.4. Analytical methods

From Table 1, it is clear that a number of methods can be used to detect promoter hypermethylation in tumors, including methylation specific PCR (MSP), real-time PCR (such as MethyLight) and bisulfite pyrosequencing. It has been suggested that a quantitative analysis, for example MethyLight, is needed for studying methylation [10,13], however, qualitative MSP has been shown to be effective and specific and does not require specific equipment [43,60]. Even though MethyLight is quantitative, there is a chance that data can differ from study to study, depending on what value is set as the 'percentage of methylated reference' (PMR) and the percentage of tumor cells present in the sample. The PMR is the value at which a given loci is declared methylated; some studies report using a PMR of >10 to declare methylation, whereas other report using a lower PMR of >4. However, it is also important to note that not all studies specifically report this value. A higher PMR results in a stricter definition of methylation, and consequently, a stricter definition of CIMP. Bisulfite pyrosequencing quantitatively measures the methylation status of several CpG sites in a given sequence, allowing the mean percentage of methylation of detected sites to be determined as a representative value [61].

4. Discussion

From this systematic review of the literature, it is clear that numerous methods and definitions are being utilized to quantify CIMP in CRC tumors. Although some of this heterogeneity may be explained by time (i.e. there have been advances in technology that have allowed for the discovery of new gene panels since CIMP was first identified), the fact remains that unlike other molecular endpoints of CRC, such as MSI, no clear biological cause or standard definition exists for defining CIMP. This makes determining the true prevalence of CIMP and comparing results across studies a challenge, and leads to other important questions. Which gene panels, marker thresholds and laboratory methods are 'best' for identifying CIMP, or does it even matter?

With respect to gene panels and marker thresholds, additional studies are necessary to assess whether CIMP-low represents a distinct phenotype in CRC, and, the debate surrounding this will likely continue until a biological cause for CIMP has been determined. In a recent review, Curtin et al. [48] conclude that *BRAF* and *KRAS* oncogene mutation status will help refine the definition of CIMP as it evolves, as it is becoming increasingly common to define the pathological and clinical features of CRC when classifying tumors, and a number of studies have shown highly methylated tumors correlate with *BRAF* mutations whereas intermediate and low methylated tumors correlate more highly with *KRAS* mutations [3,58,70,98].

It is difficult to conclude whether the difference in CIMP prevalence between studies arises because of a difference in methods, or a difference in choice of primers and/or location of methylation in the markers. MSP has a high detection signal, and subsequently, a higher prevalence of CIMP will be observed with this technique. Also, the primer/probe location of analyzed CpG nucleotides may differ between studies, and although most studies analyze methylation "around the transcription start site", no standard protocol for where to look for methylation exists. Promoter CpG islands of genes have often been reported as 'unmethylated' or 'hypermethylated', based on data of only a small number of CpG dinucleotides independent of location or the assays which have been used. It is now known that the location of core regions and the density of methylation required for gene silencing can vary per gene, therefore, a broader view than just the classical dogma of promoter CpG island methylation and gene silencing is needed to interpret data on DNA methylation, gene expression and clinico-pathological associations [99]. In the future, this may be accomplished by novel technologies that enable (semi) epigenome wide analyses of methylation profiles for specific genes.

The lack of consensus on how to quantify CIMP is a major problem. However, the biggest knowledge deficit facing this field of research is that the biological cause of CIMP in CRC remains unknown. One hypothesis is that CIMP occurs as a result of underlying genetic defects. In a recent review, Grady describes that this may include activating mutations in DNA methyltransferases or alterations in genes that control mechanisms that protect DNA from aberrant methylation [100]. It is also plausible that genetic and epigenetic abnormalities simultaneously contribute to tumor formation and progression [10,100]. Strong correlations observed between tumors with a high degree of promoter methylation and *BRAF,* and between tumors with an intermediate/low degree of methylation and *KRAS* also supports that there is causal link between methylation epigenotypes and oncogene mutation [3,58,70,98].

An alternative model gaining attention is that CIMP reflects chronic exposure to epimutagens that could then cause or accelerate cancer development through epigenetic pathways [10,100]. For the first time, large, population based studies offer a unique opportunity to elucidate such associations and link lifestyle and exposures to the phenotype. Molecular pathological epidemiology [101] now offers an opportunity to analyze environmental risk factors and disease trends in large numbers of unselected cases. With respect to CIMP, associations between

anthropometry and physical activity [55,102,103], smoking [54,87], alcohol [103,104], childhood energy restriction [105], dietary folate [103,106,107], and ethnicity [86] have been reported in case–control and prospective cohort studies. Findings from these studies offer insights on the potential etiology of CIMP in CRC. For instance, English et al. [86] reported that people of southern European origin had lower risk of colorectal cancers with CIMP than people of Anglo-Celtic origin, which may in part be due to genetic factors that are less common in people of southern European origin. Differences in ethnicity may explain why the prevalence of CIMP differs between study populations, even if the same gene panel and analytic methods were used in each. We have reported in the Netherlands Cohort Study that those exposed to severe caloric restriction early in life have a low risk of colorectal cancers with CIMP [105]. This builds on the hypothesis that methylation is an early event in CRC progression [45], and that exposures long before a given CRC event may already have implications for disease risk later in life. Such a hypothesis is supported by studies that have examined methylation patterns in normal tissue. For instance, it has been observed that CpG methylation in normal colorectal mucosa is related to advancing age [72,108–110], sex [108], race [72], rectal location [72,109], red blood cell (RBC) folate levels [109] and smoking [110]. The opportunity to pool data from large population based studies in order to improve the precision of risk estimates is a key motivation to work toward a universal definition of CIMP.

It is evident that a universal definition of CIMP is far from established and until the biological cause of CIMP is determined, this may remain a challenge. However, in order to take full advantage of the potentials of molecular pathological epidemiology, as well as develop the potential of methylation-based diagnostics and treatments for CRC, it is becoming urgent to generate discussion on this topic and aim for a consensus. To assess which technique, marker panel and threshold defines CIMP best, it will be necessary for several population based studies to test multiple techniques, maker panels and thresholds within their own set of samples.

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