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Epigenetics and Colorectal Neoplasia: the Evidence for Physical Activity and Sedentary Behavior

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Abstract

Studies demonstrate that regular physical activity and, more recently, limited sedentary behavior are associated with reduced risk of colorectal neoplasia. However, the biological mechanisms of action for physical activity versus sedentary behavior are not clear. Epigenetic variation is suggested as a potential mechanism that would allow for independent, or possibly even synergistic, effects of activity and inactivity on colorectal epithelium. We describe the evidence for epigenetic variation as a link between physical activity and sedentary behavior in colorectal neoplasia risk. There are few studies that directly evaluate this relationship. However, the growing literature describes a variety of gene targets influenced by activity that are also important to colorectal neoplasia etiology. Future studies may identify epigenetic markers with translational significance in identifying high-risk individuals or those for whom a personalized activity regimen could significantly alter the methylation signature in colon epithelial cells, and thus future risk of colorectal cancer.

Keywords

Colorectal adenoma; Colorectal neoplasms; Epigenetics; DNA methylation; miRNA; Epigenetic age; EWAS; Sedentary behavior; Physical activity

Introduction

In the USA, colorectal cancer is the third most common cancer and the second leading cause of cancer-related mortality [1–3]. The majority of colorectal cancer is believed to be sporadic, as opposed to due to inherited mutations, although estimates vary [4–6]. Strong evidence suggests that increased risk of sporadic colorectal cancer is associated with health behaviors or lifestyle factors, such as physical activity and sedentary behavior [1, 7–11]. However, the biological mechanisms influenced by physical activity versus sedentary

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behavior that may drive colorectal carcinogenesis are not yet clear [12]. Studies suggest epigenetic changes as a plausible biological mechanism linking physical activity, and possibly sedentary behavior, to colorectal cancer [13, 14] and as potential biomarkers for identifying high-risk individuals [15].

Colorectal neoplasia includes all abnormal colonic epithelium growth on a spectrum from generally benign colorectal polyps to colorectal carcinoma [16]. Colorectal cancer is unique among cancers in that there is a well-characterized, precursor lesion that can generally be removed during routine screenings, such as colonoscopy [1, 6]. However, while colonoscopy is an effective approach for identifying and removing colorectal adenomas, screening rates are lower than expected [17, 18] and it is necessary to identify complementary approaches for identifying high-risk populations. Low levels of physical activity and high sedentary behavior are associated with both increased risk of colorectal adenomas and cancer [7, 9, 10, 19–25], perhaps through inter-dependent biological mechanisms. Understanding the biological mechanisms driving observed associations between physical activity and sedentary behavior with colorectal neoplasia will provide opportunities to identify markers that may be useful in identifying individuals for whom a regimen of increased activity would significantly reduce cancer risk.

Observational Studies on Physical Activity and Colorectal Neoplasia

Physical activity is defined by the World Health Organization (WHO) as “any bodily movement produced by skeletal muscles that requires energy expenditure” [26]. In contrast, exercise is defined as a “subcategory of physical activity that is planned, structured, repetitive, and purposeful” [26]. Thus, the current review will focus on the broader measurement of physical activity, as opposed to the behavior of engaging in planned exercise. Physical activity is measured in terms of duration, frequency, and intensity [27]. Metabolic equivalent units (METs) are commonly used as a reference unit to classify activities by intensity, for which 1 MET equals the rate of energy expenditure while sitting at rest [26]. Researchers are also advocating for standard definitions of sedentary behavior and intensity of physical activity to allow comparisons across studies [27]. One suggested classification includes sedentary behavior defined as behaviors ≤1.5 METs, light activity between 1.5 and 3 METs, and moderate-vigorous ≥3 METs per physical activity [27, 28]. The American Cancer Society and WHO recommend at least 150 min of moderate intensity activity per week or 75 min of vigorous activity per week to improve overall health and reduce cancer risk [26, 29]. Studies estimate that at least 31 % of the global population is not participating in the recommended amounts of activity and rates of inactivity are nearly 20 % of the population [30]. In addition, in the USA, rates of physical activity are declining over time while sedentary behavior is increasing [31–33]. The long-term implications of this trend of decreasing activity are troubling, especially considering the consistent evidence from observational studies.

Physical activity and sedentary time are key health behaviors in colorectal cancer prevention [10, 19, 34–37]. In a recent prospective study among over 100,000 older participants in the Netherlands, higher physical activity (>90 vs. ≤30 min/ day) was associated with lower risk of colorectal cancer, particularly among women (hazard ratio (HR) 0.69, 95 % confidence

interval (CI) 0.50–0.96) [19]. In addition, the NIH-AARP Diet and Health study evaluated nearly 500,000 participants aged 50–71 years and found that men who engaged in regular physically activity had reduced risk of colorectal cancer (relative risk (RR) 0.79, 95 % CI 0.68–0.91), with a suggestive association among women (RR 0.85, 95 % CI 0.70–1.04) [10]. Furthermore, the risk of colorectal cancer consistently ranged between 0.73 and 0.88 times for the most physically active groups compared to the least in recent meta-analyses [7, 21, 36, 38–40]. These meta-analyses evaluated a variety of study designs including both case–control and prospective studies. A few studies have evaluated the relationship between physical activity and colorectal adenoma, which generally demonstrated an inverse relationship between physical activity and colorectal adenomas, although not all have identified statistically significant associations [9, 25, 41–43]. Overall, studies suggest that both colorectal cancer and adenoma risk vary by gender, which is an important factor to consider in future studies.

Similarly, emerging data also indicate that high levels of sedentary behavior, or more commonly sitting time, are associated with increased risk of cancer, independent of physical activity [44–47]. Several recent studies report that, after adjusting for physical activity, increased occupational and recreational sedentary time are associated with increased risk of colorectal neoplasia [9, 10, 19, 23, 47, 48]. Analyses from the Netherlands and NIH-AARP cohorts, discussed above, also found that higher television time and occupational sitting time, respectively, were associated with increased likelihood of colorectal cancer [10, 19]. Furthermore, Moradi et al. demonstrated that occupational sitting time was associated with increased risk of colon cancer, particularly in the distal colon, using Swedish nationwide census data [23]. In addition, Sardo Molmenti et al. also recently observed that risk of colorectal adenoma recurrence increased in older men ($n = 1730$) with the highest sedentary time compared to the lowest (odd ratio (OR) 1.47, 95 % CI 1.03–2.11), after controlling for physical activity levels [9]. A recent meta-analysis by Schmid and Leitzmann found that sitting time and time spent viewing television were associated with significantly increased risk of colon cancer (OR 1.24, 95 % CI 1.03–1.50) [48]. An additional meta-analysis by Cong et al. demonstrated increased risk of colorectal cancer for individuals reporting high levels of sedentary behavior, although evidence for differences by race or gender was equivocal [49].

Although there is strong observational evidence that physical activity and sedentary behavior influence risk of colorectal neoplasia, the biological mechanism of action for activity on carcinogenesis at the tissue or cellular level is unclear. Furthermore, it is not known if the biological mechanism of action is independent between physical activity and sedentary time, or whether the mechanisms could also lead to an antagonistic or even synergistic effect. The theories suggested for the underlying biological mechanism of action for either physical activity or sedentary time include changes to the inflammatory response, immune response and surveillance, hormone levels (such as vitamin D metabolites, insulin, and cortisol) or bile acids, gut transit time, or epigenetic modifications of genes in pathways related to immune function or known factors in carcinogenesis [12, 13, 50–53]. The purpose of this review is to summarize the small, but growing literature on the role of epigenetic mechanisms in the relationship between physical activity, sedentary behavior, and colorectal neoplasia.

Epigenetics in Colorectal Neoplasia

Epigenetic variation includes DNA modifications that do not alter the nucleotide sequence but still influence gene expression and may also be heritable [54, 55]. Epigenetic mechanisms primarily include modifications to histones, expression of non-coding RNA such as microRNA (miRNA), and variation in DNA methylation [54–57]. Unlike genetic polymorphisms, the influence of epigenetic changes on protein expression is potentially reversible and, thus, may have potential as a colorectal cancer prevention target. Molecular epidemiology has identified several target genes that are differentially methylated in normal versus neoplastic colonic epithelium [15, 55, 57]. Gene targets associated with methylation in colorectal cancer include, but are not limited to, MutL homologue 1 (*MLH1*), adenomatous polyposis coli (*APC*), the cyclin-dependent kinase inhibitor *p16*, tumor growth factor beta (*TGF-β*), *B-Raf* (*BRAF*), and *K-Ras* (*KRAS*) [15, 55]. Furthermore, the cytosine–phosphate–guanine (CpG) island methylator phenotype (CIMP) is associated with hypermethylation of CpG islands specifically near promoter regions of genes, including many of those listed above, and it is believed that 30–50 % of colorectal tumors fall into the CIMP category [58]. There is significant evidence for CIMP or methylation at other CpG loci in colorectal cancer etiology [15, 55, 57, 59]. However, few studies have evaluated the influence of physical activity on CIMP or other epigenetic genetic changes in colorectal carcinogenesis.

Epigenetics and Physical Activity

The majority of studies related to physical activity and epigenetics evaluate variation in patterns of DNA methylation at CpG sites within genes with known or hypothesized biological function [57, 59]. Global methylation status, or a quantification of total changes across the genome, which, in combination with changes to methylation status at specific loci, are common methods used to evaluate the epigenetic effects of health behaviors [54, 60]. Generally, the cancer genome is known to have a hypomethylated phenotype; however, overall lower global methylation in the genome is associated with genome stability [60, 61]. The studies of activity and global methylation presented mixed results. Among cancer-free adults (n = 161), Zhang et al. found significantly increased global DNA methylation with increasing physical activity, as demonstrated by increased global white blood cell (WBC) methylation (β=2.54, 95 % CI 0.67–4.42) among those participating in 26–30 min of daily physical activity compared to ≤5 min/day [62]. However, this study was relatively small. In contrast, in the largest study identified, Luttropp et al. observed decreased global methylation in peripheral WBCs following exercise among 1016 older adults [63]. White et al. also reported that non-Hispanic, White women (n = 647) with physical activity above the median at three time points had significantly higher levels of global methylation (β=0.33, 95 % CI 0.01–0.66), compared to women with activity levels below the median [64]. Most of the evidence, to date, comes from studies that evaluated the influence of physical activity on epigenetic variation in relation to cardiovascular disease or other chronic diseases [13, 14, 60, 65, 66] but also found changes in genes known to be associated with colorectal neoplasia etiology. Overall, these studies found that higher physical activity is associated with a cancer prevention phenotype at loci related to tumor suppressors, inflammatory cytokines, and gene transcription [58, 67, 68•]. These studies are described in detail below in addition to a review of the existing literature related to how physical activity and sedentary behavior influence

epigenetic mechanisms, and in turn how that may influence colorectal neoplasia risk. These studies are summarized in Table 1, including details on study design, methods, and results.

Epigenetic Studies of Physical Activity in Colorectal Neoplasia

There are relatively few studies that directly evaluate the role of physical activity or sedentary behavior on epigenetic variation in colorectal carcinogenesis. However, the evidence for a role of epigenetic mechanisms in risk of colorectal neoplasia is growing [59, 69–71]. Only three studies to date directly evaluated how physical activity influences DNA methylation in relation to colorectal neoplasia. Simons et al. evaluated associations between self-reported physical activity and colorectal cancer risk by the degree of DNA methylation at promoters of insulin-like growth factor binding genes (*IGFBP2*, *IGFBP3*, and *IGFBP7*) in colorectal cancer tissue [22]. Physical activity was not statistically significantly associated with colorectal cancer risk by degree of *IGFBP* promoter methylation among the 5000 participants in a case–cohort study conducted as part of the Netherlands Cohort Study [19]. However, there was a non-significant trend of reduced risk with increasing activity among individuals with three *IGFBP* promoters methylated (HR 0.90, 95 % CI (0.59–1.38), and HR 0.69 95 %, CI (0.33–1.03) for >30–90 and >90 min of activity per day, respectively; p trend=0.06) [19]. These suggestive results are important as IGF is central to inflammatory process associated with carcinogenesis [72] and should be evaluated in future studies. In addition, Gay et al. reported no association between physical activity and *MLH1* promoter methylation in a cross-sectional study of 185 colorectal cancer tumors from the EPIC-Norfolk study [73]. The *MLH1* gene has previously been associated with microsatellite instability in colorectal tumors and identified as an important factor in sporadic colorectal cancer etiology, especially serrated tumors [5, 71, 73]. However, this study was relatively small and physical activity was evaluated using self-report from an interviewer-administered questionnaire, which has a strong potential for bias. Furthermore, Slattery et al. did not find any associations between self-reported physical activity levels and CIMP status of tumors in a case–control study among adults (n = 3564) [58]. Future studies should evaluate unbiased measurements of physical activity or interventions in colorectal cancer risk or at the tissue level, which may identify novel targets for colorectal cancer prevention.

Additional studies found that physical activity may also influence DNA methylation patterns in susceptibility genes [13, 14, 62] in biological pathways relevant to colorectal carcinogenesis [66, 67, 74]. Nakajima et al. evaluated the influence of a 6-month, high-intensity walking intervention on DNA methylation in the apoptosis-associated speck-like protein containing a caspase recruitment domain (*ASC*) and *p15* tumor suppressor [60, 67]. The study found no influence of exercise on *p15*. However, exercise attenuated age-related changes in *ASC methylation* among older participants [67]. The *ASC* gene is involved in production of IL-1β and IL-18, which are members of a family of inflammatory cytokines related to colorectal carcinogenesis [60, 67, 75, 76]. In contrast, a study by Zhang et al. did not identify any associations between physical activity and *IL-6* promoter methylation, after controlling for important confounding factors [77]. Furthermore, Ren et al. compared DNA methylation profiles of regular practitioners of tai chi compared to controls, while accounting for important confounding factors such as age-related changes, smoking, and chronic disease history (n = 237) [68•]. The results of this study demonstrated that tai chi

was associated with altered methylation in genes related to cellular functions linked to carcinogenesis including DNA synthesis and repair (*RAD50*, *ERCC1*, *WRN*), nicotinamide adenine dinucleotide phosphate (*NADPH*) production related to oxidation (*G6PD*), and gene transcription (*ESR1*) [68•]. In contrast, Slattery et al. found that high levels of vigorous physical activity were not associated with methylation of *BRAF* (*n* = 1154 colorectal cancer cases and 2410 controls), which translates to the B-raf protein, a serine-threonine kinase [58]. Overall, the studies evaluating specific genes are informative, yet rather small in size (Table 1), while the study design and measurement of physical activity varied significantly. Additional studies that incorporate unbiased measures of physical activity and use an epigenome-wide approach in white blood cells as well as colorectal tissue are necessary to clarify the relationship between activity and colorectal neoplasia.

Overall, these studies support the relationship between physical activity and epigenetic variation. However, knowing which genes are specifically targeted following physical activity or sedentary behavior will improve understanding of the biological mechanisms of action and develop interventions for personalized prevention of colorectal neoplasia.

Physical Activity and miRNA Expression

Physical activity and sedentary behavior may also influence cancer risk by changing expression of miRNA with target genes known to influence colorectal carcinogenesis. Tonevitsky et al. demonstrated that, following 30 min of exercise and then recovery among eight adult males, expression of miR-21, miR-27a, and miR-18a significantly changed in whole blood [78•]. The miR-21 is known to alter expression of tumor growth factor beta (TGF-β) and platelet-derived growth factor (PDGF), which are proteins that influence regulation of cell proliferation, apoptosis, and angiogenesis [71, 78•]. Furthermore, miR-27a was upregulated following exercise, which targets expression of the *myc* oncogene, a transcription factor commonly altered in colorectal carcinogenesis [78•,79]. Finally, miR-181a has been demonstrated to modulate T cell responsiveness and may play a role in the immune response following increased activity [78•, 80, 81]. Two additional studies, by Bye et al. and Nielsen et al., evaluated the influence of physical fitness and activity on miRNA expression and also identified miR-21 as an important target [82, 83•]. In addition, Neilson et al. also reported that “chronic” exercise downregulated expression of miR-342 for which the DNA methyltransferase gene (*DNMT1*) is a target [83•]. There are no studies that directly assess the influence of physical activity or sedentary behavior on miRNA expression in normal colorectal epithelium or neoplastic tissue. However, this suggestive evidence identifies targets for evaluation in future studies.

Epigenetic Studies of Sedentary Behavior

Few studies have evaluated the role of sedentary time in DNA methylation, but none investigated colorectal cancer specifically. Alibegovic et al. evaluated the influence of 9 days of bed rest on 20 young adult males and demonstrated that inactivity significantly increased methylation of the peroxisome-proliferator-activated receptor gamma (*PPAR-γ*) in skeletal muscle [84]. In colon cancer, PPAR-γ is believed to act as a tumor suppressor and we would expect to see it upregulated by physical activity [85]. However, this study evaluated skeletal

muscle, and the results denote the importance of evaluating tissue-specific effects in future studies. Further, this study only included 20 total participants with no control group to exclude the "placebo" effect [84]. A cross-sectional study by Morabia et al. evaluated epigenetic effects of commuting by car compared to public transportation among 180 adults and found no significant difference in global LINE-1 methylation or the promoter of IL-6 in white blood cells [86•]. However, again, this pilot study was relatively small, and although commuters using public transportation are marginally less sedentary, the difference was likely not great enough to observe significant variation. Overall, evaluating the role of epigenetics as a biological mechanism of action for sedentary behavior will be necessary to understand the implications of epidemiologic studies of colorectal neoplasia etiology.

Additional Factors to Consider

Epigenetic Age

Epigenetic age represents a specific set of CpG loci that are known to predict chronological age and is an emerging area of interest in relation to cancer risk [87–89]. Epigenetic age can be calculated from deviations in DNA methylation expected with chronological age [87, 88]. Several studies have independently demonstrated that epigenetic age of colon cancer tissue is significantly different from the chronological age of the participant [87–89]. Hannum et al. reported that cancer tissue, compared to normal tissue, was approximately 40 % older for the same individual regardless of tissue type [89]. In addition, Horvath reported that methylation patterns at the loci discussed above, such as *BRAF* and *MLH1*, altered the epigenetic age of colorectal cancers [87]. Lin and Wagner also evaluated epigenetic age among over 5000 samples from 25 cancer types using both two models of epigenetic age [88]. For colorectal adenocarcinoma, epigenetic age of cancer tissue deviated significantly from chronological age and was also more highly correlated with changes at hypomethylated CpGs compared to other cancer types [88]. However, no studies have evaluated the role of physical activity on epigenetic age, yet such a study could increase understanding of the role of activity on normal aging in addition to colorectal cancer etiology.

Diet, Obesity, and Environment

There are additional factors known to influence both epigenetic variation and colorectal cancer risk that should be considered in future studies. Dietary intake of factors such as folate and processed meat also influence methylation in colorectal tissue [15, 90, 91]. Environmental factors such as ultraviolet radiation, asbestos, arsenic, and cigarette smoke are also associated with variation in DNA methylation [68]. Additional studies have also demonstrated that physical activity is associated with epigenetic variation in adipose tissue [92, 93], which may influence expression of markers or hormones with effects across multiple organ systems or tissues. Furthermore, outdoor physical activity is often associated with production of vitamin D metabolites, which are also associated with colorectal neoplasia risk as well as growing evidence of epigenetic effects [56, 94–96]. In addition, individual characteristics of participants are known to influence both colorectal neoplasia and epigenetic variation.

Gender and Anatomical Location

There are commonly observed differences by gender in associations between physical activity or sedentary behavior with colorectal neoplasia risk [9, 19]. There are several studies that suggest that the risk of colorectal adenoma or carcinoma may differ by anatomical location, especially in the proximal colon versus distal colon [38, 97]. In additional, DNA methylation patterns may differ by anatomical location, which may also support the hypothesis that biological mechanism of action or etiology of these lesions is different [97, 98]. The relationship between health behaviors or lifestyle and cancer risk is complex, which will require future studies to carefully evaluate and account for these important confounding or modifying factors.

Future Directions

Recently, researchers have begun to advocate for studies to understand the physiologic mechanisms of physical activity [99]. As summarized in Table 1, few studies directly evaluate colorectal cancer risk. However, several studies identified possible gene targets that may be important to this relationship and could be incorporated into future studies. Furthermore, studies utilizing an epigenome-wide association study (EWAS) design in large, diverse study populations or working through epigenetic consortiums will improve understanding of the impact across physiologic pathways involved in colorectal carcinogenesis. It will also be important for future studies to account for a wide range of factors in order to understand the complex relationship between health behaviors, biological mechanisms, and colorectal cancer risk. Furthermore, evaluating interactions between inherited genetic polymorphisms and epigenetic changes at the same or associated loci will be important in future research studies. Currently, most evaluate either polymorphisms or epigenetic variation, likely due to high resource requirements for omics studies [5]. However, as costs of such technology decrease and the number of consortiums evaluating these questions grows, resources may be sufficient to evaluate these complex questions. Finally, randomized trials of interventions to increase physical activity and decrease sedentary behavior will be critical to understanding the unique or overlapping mechanisms of action in colorectal cancer etiology. Results from such studies could then be used to help identify individuals at high risk for colorectal neoplasia or biological markers for colorectal cancer prevention.

Conclusions

Observational and clinical studies have provided strong evidence that physical activity and sedentary behavior influence chronic disease risk, especially colorectal neoplasia. Few studies have evaluated epigenetic mechanisms in colorectal neoplasia risk directly, while others have identified associations between activity and epigenetic variation at gene targets (such as *IGF-1*, *PPAR-γ* and *MLH1*) that may be relevant to colorectal cancer etiology. Future studies should evaluate the relationship between physical activity and sedentary behavior on colorectal neoplasia on an EWAS scale and account for factors that may also influence this relationship. As rates of physical activity decrease and sedentary time increases in daily life, it will be crucial to understand how activity alters human health.

Evaluating the epigenetic mechanisms for physical activity and sedentary time in colorectal neoplasia will improve understanding of their unique or synergistic interaction. Furthermore, no studies have yet directly evaluated the influence of activity or inactivity on colorectal adenoma, a target for colorectal cancer prevention. The challenges of this research will include identifying sufficient resources to evaluate epigenetic changes in large epidemiologic studies, harmonizing physical activity measures across studies, and staying up-to-date with the technological advances in measurement of epigenetic variation in the lab. Such studies are important, however, as the research may identify novel, modifiable markers with translational significance as targets for personalized prevention of colorectal cancer. Epigenetic markers may also identify individuals at high risk for colorectal neoplasia, for whom a complementary regimen of increased exercise and reduced sedentary time could significantly alter their epigenetic signature and thus colorectal cancer risk.

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Table 1

Selected studies evaluating physical activity, sedentary behavior, and epigenetic modifications relevant to colorectal cancer

Authors (year)	Study design	Number	Biospecimens	Activity measurement	Outcome/ targets identified
Physical activity					
Simons et al. (2014) [22]	Case–cohort	5000	Colorectal tissue	Self-administered	↓, Colorectal cancer risk with 3 *IGFBP genes* methylated
Gay et al. (2011) [73]	Cross-sectional	185	Colorectal tissue	Interviewer-administered	No association with *MLH1* methylation
Slattery et al. (2006) [78]	Case-control	1154 Cases, 2410 Controls	Colorectal tissue	Interviewer-administered	No association with CMP or *BRAF*
Luttrop et al. (2013) [63]	Cohort	1061	White blood cells	Self-administered	↑ Physical activity associated with ↑global methylation
Tonevitsky et al. [79•]	Intervention	8	White blood cells	Timed exercise	Physical activity led to ↑ miR-21 and miR-27a, ↓miR-181a
Zhang et al. (2012) [53]	Cross-sectional	165	White blood cells	Self-administered	No change in global (LINE-1) or *IL-6* promoter methylation
Bye et al. (2013) [83]	Cross-sectional	720	Whole blood	Self-reported	No association with miR-21 expression
White et al. (2013) [64]	Cohort	647	Whole blood	Computer-assisted	Physical activity above median associated with ↑global methylation
Ren et al. (2012) [68•]	Cross-sectional	237	Saliva	Self-administered	↓ Methylation at *Rad50_2, 17p_7, G6PD*
Nielsen et al. (2014) [84•]	Intervention	32	Plasma	60 min, 5 times per week sessions over 12 weeks	↓ miR-21 expression
Sedentary behavior					
Alibegovic et al. (2010) [85]	Intervention	20	Skeletal muscle	9 days of bed rest	↑ *PPAR-γ* methylation
Morabia et al. (2012) [87•]	Cross-sectional	180	White blood cells	Self-administered	No association with Global LINE-1 or IL-6 promoter methylation