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REVIEW

DNA methylation-based variation between human populations

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Abstract Several studies have proved that DNA methylation affects regulation of gene expression and development. Epigenome-wide studies have reported variation in methylation patterns between populations, including Caucasians, non-Caucasians (Blacks), Hispanics, Arabs, and numerous populations of the African continent. Not only has DNA methylation differences shown to impact externally visible characteristics, but is also a potential biomarker for underlying racial health disparities between human populations. Ethnicity-related methylation differences set their mark during early embryonic development. Genetic variations, such as single-nucleotide polymorphisms and environmental factors, such as age, dietary folate, socioeconomic status, and smoking, impacts DNA methylation levels, which reciprocally impacts expression of phenotypes. Studies show that it is necessary to address these external influences when attempting to differentiate between populations since the relative impacts of these factors on the human methylome remain uncertain. The present review summarises several reported attempts to establish the contribution of differential DNA methylation to natural human variation, and shows that DNA methylation could represent new opportunities for risk stratification and prevention of several diseases amongst populations world-wide. Variation of methylation patterns between human populations is an exciting prospect which inspires further valuable research to apply the concept in routine medical and forensic casework. However, trans-generational inheritance needs to be quantified to decipher the proportion of variation contributed by DNA methylation. The future holds thorough evaluation of the epigenome to understand quantification, heritability, and the effect of DNA methylation on phenotypes. In addition, methylation profiling of the same ethnic groups across geographical locations will shed light on conserved methylation differences in populations.

Keywords DNA methylation · Phenotypic variation · Ethnic groups · Human populations

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Introduction

DNA methylation

Epigenetics has been defined as the study of the effects of structural and chemical modifications to chromatin and its component proteins and DNA (Murrell et al. 2005). DNA methylation is the first epigenetic modification identified on DNA. It is an epigenetic mark of paramount importance for normal development in the human genome. The role of DNA methylation in differential regulation of gene expression, imprinting (differential gene expression depending on the parent-of-origin) and gene silencing have helped to understand the relationships between genotype and contribution of epigenome to phenotype (Oakes et al. 2016; Rotti et al. 2015; Stefansson et al. 2015). Methyl-cytosine is the product of covalent attachment of a methyl group to a cytosine residue of the DNA sequence. DNA methylation occurs chiefly in CpG dinucleotides; however, methylation elsewhere has been documented (Lister et al. 2009; Schultz

et al. 2015; Pinney 2014; Yan et al. 2011). Residing within the human genome are approximately 30 million CpG dinucleotides which are unmethylated, hemi-methylated or abundantly methylated; varying according to region on chromosome, alleles, type of cell or phase of development (Rienius et al. 2012; Tammen et al. 2013). DMRs (differentially methylated regions) are contiguous genomic regions that vary between phenotypes. These may occur throughout the genome, but have also been identified around the promoters of genes, intragenically as well as in intergenic regulatory regions (Peters et al. 2015; Rakyan et al. 2011; Spilianakis et al. 2005). Differentially methylated regions could be tissue specific (tDMRs), cancer specific (cDMRs) or age specific (aDMRs) (Rakyan et al. 2011), or as the recently coined, population-specific (pop-DMRs) (Heyn et al. 2013; Hernando-Herraez et al. 2015). Pop-DMRs are mostly associated with several histone modifications and transcription factor binding sites. Pop-DMRs also occur in genes related to susceptibility to different diseases and environmental factors (Hernando-Herraez et al. 2015). Since methylation patterns of DMRs differ, DMRs have been used as dynamic, targetable, candidates for biomarkers in medical and forensic research (Kader and Ghai 2015; Levenson and Melnikov 2012; Rakyan et al. 2008; Wan et al. 2015). For a detailed review of applications of DNA methylation in forensics, please refer to Kader and Ghai (2015).

The methylation reaction is mediated by methyltransferases (DNMTs), namely, DNMT1, DNMT3A, DNMT3B, and DNMT3L, which introduce a methyl group derived from *S*-adenosylmethionine (SAM) onto the C5 position of cytosine residue. DNMT1 acts on hemi-methylated DNA at replication foci which is recognized by nuclear protein 95 (NP95) or Ubiquitin-like with PHD (plant homeodomain) and ring finger domain 1 (Uhrf1). The enzyme maintains methylation by copying methylation marks from the parental strand onto newly synthesised daughter strands (Bostik et al. 2007; Seisenberger et al. 2013). DNMT3A and DNMT3B are de novo methyltransferases, which act on unmethylated DNA and establish methylation patterns during early development (Chen and Riggs 2011). DNMT3A/DNMT3B also contributes to the maintenance of DNA methylation patterns. Methylation at most imprinted loci also requires the activity of DNMT3L (Chen and Riggs 2011; Kaneda et al. 2004).

Global DNA demethylation is important for establishing pluripotent states in early embryos and for erasing parental-origin of specific imprints in developing primordial germ cells. Local DNA demethylation occurs in tissue-specific genes throughout development and cellular differentiation (Frank et al. 1991; Hill et al. 2014). DNA demethylation occurs via two processes, namely, passive and active. Loss of maintenance methylation activity results in passive DNA demethylation during several cycles of DNA replication. Active DNA demethylation would lead to the removal of 5-meC in a replication-independent manner. This could occur through the enzymatic deamination of 5-meC to thymine, coupled with G/T mismatch repair by DNA glycosylases (Kress et al. 2001; Zhu 2009). Alternatively, the TET family of oxygenases catalyse the oxidation of 5-meC to 5-hydroxy methyl-cytosine; leading to DNA demethylation.

DNA methylation changes in early development

Erasure of DNA methylation patterns of the gametes (oocyte and sperm) (Gannon et al. 2014) in the zygote occurs immediately after fertilization. The paternal genome undergoes a rapid demethylation before the first cell division cycle, except paternally imprinted genes, heterochromatin, around centromeres and some repetitive elements (Gluckman et al. 2009; Nafee et al. 2007). The maternal genome undergoes gradual passive demethylation during the several cell divisions leading towards blastocyst formation due to a lack of maintenance of methylation in the zygote (Geiman and Muegge 2010; Mayer et al. 2000; Oswald et al. 2000; Pan et al. 2012; Rougier et al. 1998). Embryogenesis up until the blastocyst stage is crucial in determining the epigenetic marks needed for later development (Geiman and Muegge 2010). Following the demethylation steps, there is an increase in de novo methylation through the activity of de novo methyltransferases DNMT2A and 3B (Geiman and Muegge 2010; Okano et al. 1999). DNA methylation is re-established and is responsible for cell lineage-dependent differentiation leading to the formation of each of the fully differentiated cells of the offspring (Fig. 1; Borgel et al. 2010; Marsit 2015).

The epigenome is highly sensitive and most vulnerable to the environment during early stages of development, because the genome methylation pattern is established during this stage and the DNA synthetic rate is very high in the early embryo. Early life events cause changes in the epigenome that are associated with increased susceptibility to chronic non-communicable diseases (Gluckman et al. 2009). Studies have indicated that prenatal factors like periconceptional and prenatal socioeconomic status (SES) and childhood SES may have an influence on DNA methylation (King et al. 2015).

Several studies have shown that racial differences in gene-specific DNA methylation levels are even present at birth (Adkins et al. 2011; Terry et al. 2008). Lower global levels of DNA methylation among healthy middle-aged African American women relative to Caucasians were found by these groups. A proportion of these differences in rates of incidence undoubtedly can be ascribed to differences in the frequencies of SNP and DNA repeat alleles (Ashktorab et al. 2003; Pernick et al. 2003), maternal diet

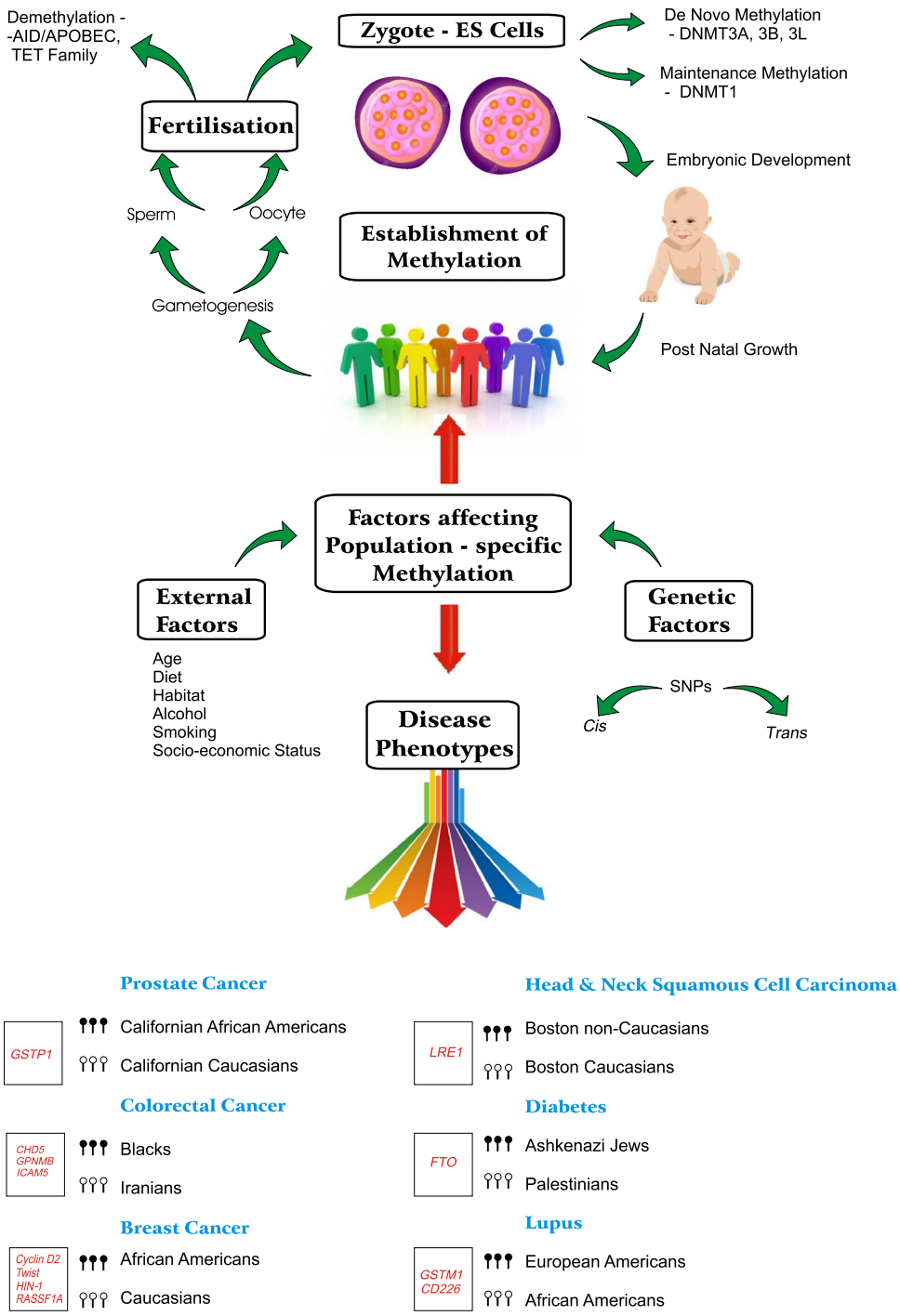

Fig. 1 Factors that shape the human methylome set their mark during early embryonic development and lead to differential methylation patterns in populations. Establishment of methylation is mediated by DNMT1, DNMT3A, DNMT3B, and DNMT3L. Demethylation of the gametes (oocyte and sperm) in the zygote occur immediately after fertilization, mediated by spontaneous cytosine deamination by AID/APOBEC enzymes or the TET family of oxygenases that catalyse oxidation of 5mC to 5-hydroxy-mC. DNA methylation differences among populations are due to varying environmental and genetic factors. These factors may lead to disease phenotypes which are shown to differ between populations (represented by various *colours*). The figure represents an example of one study of each disease discussed in text. Prostate cancer (Enokida et al. 2005); colorectal cancer (Mokarram et al. 2009); breast cancer (Mehrotra et al. 2004); head and neck squamous cell carcinoma (Hsiung et al. 2007); diabetes (Toperoff et al. 2015); lupus (Coit et al. 2013). *Dark lollipops* indicate high methylation levels of the disease-associated gene in that particular population/ethnic group, whereas *light lollipops* indicate low methylation levels (colour figure online)

(e.g., availability of methyl donors, folate supplementation) and behavioral/socioeconomic factors (Siahpush et al. 2010; Subramanyam et al. 2013; Surgeon General Report 1998).

Epigenetic differences between human ethnic groups are also dependent on social factors which are outlined during birth. Newborns with Black and Hispanic fathers had lower methylation of the imprinted genes *Igf2* and *H19* DMRs when compared to newborns with Caucasian fathers (King et al. 2015). This study by King et al. (2015) revealed that ethnicity, income and education-based differences exist in DNA methylation at *Igf2, H19* and *MEG3* imprinted genes DMRs. No significant differences were noted for *PEG3, PEG10/SGCE/PLAGL1, PEG1/MEST, MEG3-IG* or *NNAT* DMRs.

An interesting study observed that individuals exposed to famine in utero, and their same sex siblings displayed variation in DNA methylation at the *Igf2* imprinted domain.

The change could predict chronic diseases in later life of both mother and offspring (Heijmans et al. 2008; Painter et al. 2008). Thus, aberrantly established epigenetic marks that regulate gene expression have been linked to diverse outcomes as cancer, asthma, birth weight, and hormonal and metabolic profiles (Salam et al. 2012; Hoyo et al. 2014; Veenema 2012). A recent study by Straughen et al. (2015) compared methylation levels of *Igf1* levels in Black and non-Black neonates and discovered that high methylation levels of *Igf1* in Black neonates may play a central role in variation of expression, leading to lower birth weights and hence onset of disease later in life.

Heritability of methylation patterns

A number of methylated loci are protected and are carried over through zygote divisions and generation of PGCs (primordial germ cells), thus providing potential for DNA methylation patterns to be transmitted to the next generations (Feng et al. 2010; Jiang et al. 2013; Potok et al. 2013; Seisenberger et al. 2012). It is within this time of reprogramming that every gene acquires a particular DNA methylation pattern (Hajkova et al. 2002; Tammen et al. 2013).

However, DNA methylation heritability is closely related to genetic effects (McRae et al. 2014), and it is difficult to distinguish true epigenetic inheritance from genetic inheritance. Heritability of DNA methylation differs according to site and density of methylation. Rowlatt et al. (2016) found that DNAm sites in regions of low-CpG density were more methylated and were more likely to be significantly heritable when compared with DNAm sites in regions of high-CpG density.

van Dongen et al. (2016) studied DNA methylation levels in peripheral blood (411,169 autosomal sites) using the Illumina 450k array. The study was based on a large population-based twin cohort ($n = 2373$), also including family members (parents $n = 212$; siblings $n = 16$) and spouses of twins ($n = 3$). This study found that the average genome-wide heritability of methylation was 0.19. Common genetic variants could account for about 7% of variable DNA methylation patterns, and explained approximately 37% of the total heritability of methylation levels. In addition, at 18% of the 450k targeted sites, over nearly 100% of the heritability was explained by common single-nucleotide polymorphisms (SNPs). Numerous methylation sites were identified where the impact of environmental or stochastic influences on DNA methylation increased with age. For example, at the cg22178392 site in the *TNIP1* gene in blood, heritability of DNA methylation decreased from 0.54 at age 25 to 0.39 at age 50. Interestingly, those sites with high heritability were enriched in CpG islands and DNase I hypersensitive sites and methylation sites showing significant interaction between environmental effects and age were most strongly enriched in CGI shores. The effect of smoking was also examined and the groups found that smoking-associated sites were on average moderately heritable (0.5). Some sites overlapped with age, for example, methylation level at cg12803068 in *MYO1G* had a heritability of 0.91 at age 25 and a heritability of 0.71 at age 50. This study shows the presence of both genetic and environmental effects on heritability of methylation. These factors are further elaborated later.

Currently, extensive genome-wide research aims at identification of tissue- and gene-specific variations in DNA methylation that may contribute to phenotypic differences between populations (Bell et al. 2011; Fraser et al. 2012; Heyn et al. 2013; Lam et al. 2012). Undoubtedly, DNA methylation has had a substantial impact on the development of human genome sequences. With new advances in technology, other than differentiating and finding links between individuals based on gender, age and diseases, great progress has been made in establishing the contribution of differential methylation to natural human variation. The present review discusses variation in DNA methylation patterns between various populations (pop-DMRs) of the world and its potential implications. The review is divided broadly into subsections starting with disease associated variation in DNA methylation among human population and non-disease associated variation, followed by genetic and environmental factors affecting population-specific DNA methylation variation.

Variable DNA methylation patterns between populations

Disease-associated variations of DNA methylation patterns between populations

Over the past two decades, ample efforts have been made to understand the underlying determinants in racial health disparities. Research of different racial/ethnic groups has painted a picture of distinguishable patterns of DNA methylation, gene expression and the onset of numerous cardiovascular diseases, as well as cancers (Adkins et al. 2011; Edwards et al. 2005; Heyn et al. 2013; Tost 2010; Kuzawa and Sweet 2009). There is indeed evidence that loss of gene expression due to promoter hypermethylation has clinical implications in some cancers. Hypermethylation-induced loss of expression affords a cell with growth promoting features; such as evasion of apoptosis, insensitivity to antigrowth signals, unlimited replicative potential as well as self-sufficiency in growth signals (Herman and Baylin 2002; Laird 2003; Mehrotra et al. 2004; Widschwendter and Jones 2002). The introduction of genome-wide DNA methylation analysis comparing tumorous and

non-malignant tissues has resulted in the discovery of many regions that undergo aberrant methylation during carcinogenesis, and these regions show differential DNA methylation patterns between ethnicities (Jain et al. 2013; Tost 2010; Ma et al. 2013; Wild and Flanagan 2010). For example, Tomii et al. (2006) developed a methylation-specific PCR (MS-PCR) method and found that methylation of the promoter region of *IGTBP-3* gene (insulin-like growth factor binding protein-3) in malignant mesothelioma were lower in patients from the USA than patients from Japan (*IGTBP-3* was methylated in 15% of USA samples compared to methylation in 75% of Japanese counterparts). This was suggestive of the presence of ethnic differences in the *IGFBP-3* methylation status.

Squamous cell carcinomas

Cancers of the lung are said to be one of the leading causes of cancer-related mortalities worldwide (Molina et al. 2008; Tost 2015) and DNA methylation has been found to have a significant effect in the onset and prognosis of the cancer, especially in Caucasians. Piyathilake et al. (2003) evaluated the methylation status of squamous cell carcinomas (SCC) of the lung, as well as the associated uninvolved epithelial hyperplasia (EH) and bronchial mucosa (UBM) in Caucasians and African Americans, using an antibody specific for 5-methylcytosine (5-mc). The study found that African Americans displayed similar methylation in SCC, EH and UBM whilst for the Caucasians, methylation levels of SCC were significantly lower than the EH and UBM. Thus, DNA methylation played a significant role in the onset of SCC in Whites, but not African Americans (Piyathilake et al. 2003).

Head and neck cancer is the sixth most common cancer in the world. Each year, there are approximately 600,000 reports of new cases, with approximately 90% being squamous cell carcinoma (Parkin et al. 2005; Virani et al. 2015). Gene-specific DNA methylation has been documented as a contributor to the molecular heterogeneity of head and neck squamous cell carcinoma (HNSCC). Numerous markers have been proposed as biomarkers of prognosis and/or diagnosis of the disease including *NDN* and *CD1A* (Demokan and Dalay 2011; Virani et al. 2015). Hsiung et al. (2007) studied the role of DNA methylation in HNSCC, which is also commonly associated with exposure to alcohol, smoking, dietary folate and human papillomavirus. The group examined global methylation levels of a long interspersed nuclear element repeat region, *LRE1*, in whole blood, using an altered version of combined bisulfite restriction analysis and found a substantial 1% ($p < 0.03$) increase in methylation levels in non-Caucasian subjects as opposed to Caucasians in Boston. This was suggestive of non-Caucasians having a higher risk of the disease. This study found that in patients, both low dietary folate and variant *MTHFR* C677T genotype showed decreased *LRE1* methylation when compared to healthy controls; and in healthy controls, antibody response to human papillomavirus 16 (HPV) was correlated with higher methylation.

Prostate cancer

In the United States, prostate cancer is among the most common malignancies in men. It has become evident that not only do prostate cancer cells carry a range of genetic defects such as mutations, deletions and amplifications, but these cells also carry epigenetic defects, especially changes in DNA methylation. Transcriptional silencing by aberrant DNA methylation of CpG-rich promoter regions is largely responsible for epigenetic inactivation of genes in prostate cancer cells (Enokida et al. 2005; Hsing et al. 2000; Kwabi-Addo et al. 2010). Numerous studies have observed a higher incidence and mortality rate in African Americans when compared to Caucasians, whereas the lowest incidence has been reported in Asians (Hsing et al. 2000; Jemal et al. 2004; Watanabe et al. 2000). Several reports have shown that increased methylation of the *GSTP1* promoter (π-class glutathione *S*-transferase) is pivotal in gene expression in prostate cancer (Jerónimo et al. 2002; Maldonado et al. 2014). Enokida et al. (2005) investigated methylation levels of the promoter of *GSTP1* between African Americans, Caucasians (from San Francisco, California) and Asians (of Japan) and found that *GSTP1* hypermethylation is a sensitive diagnostic marker for African Americans. MS-PCR was performed using two primer sets termed MSP-A and MSP-B. The primer sets targeted 15 CpG sites and Sp1 (specificity protein 1) sites. Highest positive methylation levels were displayed in 79% of African-American samples for MSP-A and 68.2% for MSP-B. In contrast, 68.8% of Caucasians showed methylation for MSP-A, and 61% showed methylation for MSP-B, whereas Asians showed 75.3% for MSP-A and 67.1% for MSP-B. This corresponded with a higher hazard ratio of 13.361 in African Americans when compared to Caucasians and Asians; 3.829 and 8.603, respectively. Expression of *GSTP1* mRNA was downregulated by hypermethylation of the gene.

Similarly, Kwabi-Addo et al. (2010) examined the methylation status of prostate cancer-related genes; *GSTP1, AR, RARβ2, SPARC, TIMP3,* and *NKX2-5* in African American and Caucasian American populations. The study also found higher methylation levels of the genes such as *NKX2-5* and *TIMP3* in African Americans compared to Caucasians, with p values of 0.008 and 0.039, respectively. These differing methylation levels were not attributed to any underlying genetic variants, but rather a reflection of varying lifestyles, environmental exposure, diets or susceptibility to methylation (Kwabi-Addo et al. 2010).

In contrast to studies detecting a higher incidence and mortality rate in African Americans, Das et al. (2006) examined the methylation-directed silencing of the prostate cancer tumour suppressor gene *TMS1/ASC* (target of methylation induced silencing; also called *PYCARD*) and found prevalence of *TMS1* gene methylation to be lower in Caucasians (62.2% prevalence) than African Americans (67.7% prevalence); with a corresponding (age-adjusted) higher hazard ratio of 7.6 in Caucasians and lower hazard ratio of 1.1 in African Americans. Das et al. (2006) found a significant difference in methylation within each population's prostate cancer patients and healthy controls. In Whites, prevalence for gene methylation was 62.2% for patients and only 22.7% in healthy controls, whereas in Blacks, there was similar prevalence of 66.7% for patients and 58.3% for controls. While the causative factors for these ethnic-specific variations in prostate cancer gene methylation are not yet fully established, they are partly attributed to diet, lifestyle and socioeconomic factors (Fang et al. 2005; Woodson et al. 2004). A more recent study by Graham-Steed et al. (2013) showed similar mortality rates amongst Africans and Caucasians in equal-access health care systems, implying that racial/ethnic differences in mortality rates of African American and Caucasian men do not take into account socioeconomic and clinical factors.

Colorectal cancer

In colorectal cancer research, Mokarram et al. (2009) used MSP to conduct a population-to-population comparison of promoter methylation of several candidate genes in Blacks and Iranians. The study revealed similar methylation profiles between the populations for the *SYNE1, MMP2, PTPRD, RNF182, RET*, *EVL, CD109, APC2,* and *STARD* genes and significantly different methylation profile between Blacks and Iranians for; *CHD5* (chromodomain helicase DNA binding protein V; 78% Blacks vs. 47% Iranians), *GPNMB* (Glycoprotein Transmembrane; 100 vs. 89%), *LGR6* (leucine-rich repeat-containing G protein coupled receptor 6; 49 vs. 31%), and *ICAM5* (intercellular adhesion molecule V; 40 vs. 7.5%) with three genes showing most statistically significant differences (*CHD5, GPNMB,* and *ICAM5*) accounting for the higher incidence and aggressiveness of the disease in Blacks. For both populations, methylation was found to be dependent on patient gender for four genes; higher methylation for *RET* and *APC2* was observed in males, whereas higher methylation for *STARD8* and *PTPRD* was observed in females. Similarly for both populations, methylation of four genes was found to be influenced by site, namely, *CHD5, LGR6, CD109,* and *ICAM5* which displayed lower methylation levels in the distal colon. The authors investigated the effect of confounding factors such as site, differentiation and stage of cancers among the two populations. They found that site and differentiation were confounding factors for the *ICAM5* gene but not for *CHD5* and *GPNMB*. Logistic regression models were developed using forward selection with the genes as dependent factors and populations, site, stage, and differentiation as independent factors. For all three regression models, DNA methylation remained the significant factor for disease progression in Blacks.

Breast cancer

In addition to skin cancer, breast cancer is the most commonly diagnosed cancer among American women. The malady has a high morbidity and mortality among women worldwide. According to a recent report by Breastcancer.org, in women under 45, breast cancer is more common in African American women than Caucasian women. African American women are more likely to die of breast cancer when compared to Hispanic, Asian and Native American women. African American women exhibit several negative prognostic factors such as early age of diagnosis, unique mutations in some oncogenes (*BRCA1* and *p53*) and oestrogen and progesterone receptor negativity (ER^-/PR^-) (Adkins et al. 2011). In an assessment of ER/PR positive and negative invasive ductal carcinomas (Mehrotra et al. 2004), several oncogenes displayed similar methylation patterns in all groups except African-American women less than 50 years of age with ER-/PR- tumors. Among them, there was considerably higher promoter methylation of four genes [*Cyclin D2*—64% in African Americans vs. 19% in Caucasians ($p < 0.0001$); *Twist*—67% in African Americans vs. 16% in Caucasians ($p < 0.0001$), *HIN-1*—79% in African Americans vs. 19% in Caucasians ($p < 0.0001$); *RASSF1A*—76% in African Americans vs. 29% in Caucasians ($p < 0.0001$) and *RAR-β*—40% in African Americans vs. 8% in Caucasians ($p = 0.01$)]. These genes were involved in apoptosis and tumour suppression. To assess biological significance of hypermethylation, quantitative reverse transcribed PCR was used to measure *HIN-1* gene expression. Hypermethylation of *HIN-1* was indeed associated with mRNA expression in the tumors since 10 of the 14 tested tumours that were methylated for the *HIN-1* gene did not show detectable expression of *HIN-1* mRNA (Mehrotra et al. 2004).

Zhang et al. (2013) assessed the methylation status of the *PTEN* gene (tumour suppressor) in a Chinese population. A reduced *PTEN* expression rate occurs in over 80% of breast cancer cases. Aberrant increase in methylation of the promoter regions is a critical event in breast tumorigenesis and tumor progression (Bose et al. 2002; Widschwendter and Jones 2002; Zhang et al. 2013). In their study of Chinese women, *PTEN* methylation was found in

31.1% of breast cancer patients, 64.3% of which exhibited a loss of *PTEN* expression (Zhang et al. 2013). Sadeq et al. (2011) analysed *PTEN* promoter methylation in breast cancer patients of Iran. *PTEN* promoter methylation was in 37 of 53 tumor tissues, whereas methylation was not detected in 20 healthy counterparts. Promoter methylation was found in patients with heterozygote mutation in the *PTEN* gene. Yari et al. (2015) also studied breast cancer among a Kurdish population from Iran. The study found a 6% frequency of *PTEN*-methylated (MM) genotype in non-cancer patients (controls) and a 41.7% frequency in patients. Furthermore, the authors found that in the presence of the *PTEN* MM genotype, there was as 3.1-fold susceptibility to onset of breast cancer when compared to the UU (Unmethylated) genotype in the Kurdish population. In addition, in the presence of *PTEN* M allele, the risk of breast cancer was 2.71-fold compared to the presence of U allele (Yari et al. 2015). These studies emphasised the significant role of promoter hypermethylation in the progression of breast cancer.

The national incidence rate of breast cancer in Uruguay is 90.7 per 100,000 women per annum (Globocan 2012), making the illness one of the most common in the country. Cappetta et al. (2015) determined global leukocyte DNA methylation levels of 42 subjects with melanoma and 46 healthy controls, and 86 women with breast cancer and 92 healthy female controls. All subjects were from Uruguay. Methylation levels in melanoma and breast cancer patients were significantly lower than the methylation levels of healthy controls. Methylation levels of melanoma patients and controls were 2.54 and 2.79%, respectively; levels of breast cancer patients and controls were 2.33 and 2.77%, respectively. In contrast to several studies, no relationship was observed between global DNA methylation levels, and 59 biallelic SNPs spaced along the 22 autosomes, which showed large difference in allele frequency between ancestral populations (>0.5), were used in the study as ancestry informative markers (AIMs) to estimate admixture proportions in the study common genetic variants, such as C677T in *MTHFR*, rs4665777 in *DNMT3A* or rs16942 and rs8176092 in *BRCA1* in the breast cancer study, were observed. The Uruguayan population has a tri-hybrid parental contribution of European, African, and Native Americans. To understand the relationship between DNA methylation and ancestry, methylation levels of CpGs within a window of 100 kb surrounding each AIM were measured in populations that were closely related to parental populations of the Uruguayan samples. For this, methylation data were obtained from 96 African Americans, 96 Caucasians and 96 Han Chinese Americans which were used previously in the Heyn et al.'s (2013) study. Methylation levels in promoters, intergenic regions as well as gene bodies showed significant differences between the three populations; with levels of methylation being similar between African Americans and Han Chinese Americans, but both significantly higher than methylation levels observed in Caucasians. Such results showed that differences also occurred in parent populations; and thus, the tri-hybrid structure of the admixed Uruguayan population could partially explain the observed methylation patterns between the groups. Furthermore, a correlation analysis was performed to decipher whether individual ancestry has an effect on global DNA methylation status. This analysis found an indirect relationship between the African ancestral component and levels global DNA leukocyte methylation in breast cancer patients, while no relationship was observed for melanoma patients or any of the healthy controls (Cappetta et al. 2015).

Diabetes

It is evident that most research associating DNA methylation and disease focuses on cancer; however, aberrant methylation has been linked to a growing number of human ailments including Parkinson's disease, Alzheimer's disease, asthma, atopic dermatitis, inflammatory bowel disease, rheumatoid arthritis, and diabetes (Calvanese et al. 2009; Lohmueller et al. 2003; Rienius et al. 2012; Tost 2010). In diabetes research, altered DNA methylation levels were reported in insulin-responding and insulin-secreting tissues of diabetes patients (Volkmar et al. 2012; Yang et al. 2012). Studies have provided evidence that the prevalence of diabetes is 64% higher in Middle-Eastern and North African countries compared to European countries (Alhyas et al. 2012; Badran and Laher 2012; Danaei et al. 2011). Toperoff et al. (2012) studied diabetes in Ashkenazi Jews and showed that a CpG site in the first intron of the *FTO* gene (fat mass and obesity-associated) showed significant hypomethylation of 3.35%; $p = 0.000021$ in patients relative to the healthy controls. This specific methylation pattern occurred prior to the onset of type 2 diabetes manifestations. The same group of researchers' found significant variations in DNA methylation levels of peripheral blood leukocytes (PBLs) between East Jerusalem Palestinian and Israeli Ashkenazi Jew diabetes patients (Toperoff et al. 2015). For both cohorts, an inverse association between diabetes and PBL methylation was found, which occurred independently of body mass index, lymphocyte-to-granulocyte ratio or gender. The methylation, however, did decrease with age. Methylation levels of healthy controls in both cohorts were higher at young ages which decreased as age progressed. In contrast, Palestinians were hypomethylated compared to their Jewish counterparts across most of the age distribution, suggestive of a mechanistic link between the earlier hypomethylation of the Palestinians and the higher diabetes prevalence characteristic

of this population. Consistent with earlier onset of diabetes in Palestinians, this population attained accelerated demethylation and average methylation levels of diabetes patients about a decade earlier than Jews. The authors tried to determine the influence of several factors which could have given rise to the observed diabetes-related methylation. After extensive sequencing of DNA containing the diabetes-related methylation and sequence variation, *cis*-sequence-influenced methylation was not likely to account for the observed diabetes-related methylation. Early development patterning of inter-individual differences shared by disease-related and unrelated tissues, or blood-based mechanisms could have possibly accounted for the variable methylation. The possibility of differential white blood cell composition as the underlying mechanism for variable methylation was also tested and rejected. The interrogated CpG site was found to be independently associated with methylation. However, other blood-based mechanisms could have been possible, such as example differential activity states of circulating leukocytes between diabetic and non-diabetic individuals, but this requires further investigation (Toperoff et al. 2015).

Chambers et al. (2015) investigated differences in diabetes-associated DNA methylation patterns between Indian Asians and Europeans using epigenome-wide association studies. Over a period of 8.5 years, 7.6% fewer Europeans developed diabetes, compared to Indians. When stratifying for differences with age- and sex-matched controls, the incidence of diabetes was 3.1 times lower among Europeans than Indian Asians, and remained lower in Europeans even after considering physical activity, genetic history of diabetes and adiposity. Five markers associated with diabetes were detected, namely, *TXNIP*, *ABCG1, SOCS3*, *PHOSPHO1,* and *SREBF1*. Combined results of these loci showed similar results with lower methylation in Europeans. The reasons underlying the variances in methylation at these 5 loci before type 2 diabetes onsets were not known. However, *TXNIP* expression is sensitive to glucose concentration, which correlates with aberrant *TXNIP* methylation being an early marker for impaired glucose homoeostasis. In addition, DNA methylation at the other four markers; *ABCG1, SOCS3*, *PHOSPHO1* and *SREBF1,* was associated with BMI, waist circumference as well as insulin concentrations. Among 1932 normoglycaemic Indian Asians, future risk of type 2 diabetes incidence was up to four times higher among obese and overweight Indian Asians, but not among normal weight individuals. Methylation of the loci near these five genes was associated with body mass index which were lower in Indian Asians; and glucose concentrations and waist:hip ratios; both of which were higher in the Indian Asian population than the Europeans. In the test to determine if there was a correlation between DNA methylation in blood and methylation in a metabolically relevant tissue, for 175 samples from obese Europeans, a relation was noted between methylation in peripheral blood and methylation in the liver at the *SOCS3* ($p = 5.3 \times 10^{-5}$) and *TXNIP* ($p = 0.02$). In this group of obese Europeans, evidence for association between methylation and gene expression of *PHOSPHO1* and *SOCS3* was found in blood; DNA methylation was also found to be associated with the gene expression of *SREBF1* and *ABCG1* among both populations in blood. In the liver, methylation was associated with the expression of *TXNIP.* These findings show that DNA methylation might be a biomarker of metabolically unfavourable patterns of adiposity and insulin resistance.

Other autoimmune disorders

Much evidence for the participation of DNA methylation in autoimmune diseases has surfaced over recent years. For instance, studies have shown that the role of DNA methyltransferase activity (Wiley et al. 2013) and alteration of DNA methylation levels in $CD4^+$ T cells could play a significant role in the onset and clinical presentation of systemic lupus erythematosus (SLE). The disease is deemed to be less severe in European Americans compared to African Americans (Coit et al. 2013; Somers et al. 2014). Wiley et al. (2013) analysed *DNMT1, DNMT3A,* and *DNMT3B* in peripheral blood mononuclear cells obtained from African American and European American lupus and non-lupus female subjects. In patients with SLE, the researchers found no significant differences in mRNA expression levels of *DNMT1* when compared to the age-matched controls. However, lupus patients were found to display a higher expression level of *DNMT3A* when compared to healthy counterparts; and European American lupus patients had significantly lower levels of *DNMT3A* expression when compared to African American lupus patients. This study revealed the methylation status of over 27,000 CpG sites in promoter regions of ~15,000 genes of which 105 sites were hypermethylated and 236 sites were hypomethylated in female lupus patients. It is possible that early life environmental influences, such as exposure to dangerous agents, and diets, infections could partially account for shifts in *DNMT* expression and antibody production, as several studies have proved (Balada et al. 2007; Ehrlich 2003; Wiley et al. 2013).

Coit et al. (2015) studied variation of DNA methylation in naïve $CD4^+$ T cells in healthy European American and African American populations and compared results to matching SLE counterparts. The group identified distinct methylation profiles between the two populations using the Illumina Infinium HumanMethylation450 microarray. Three-hundred and seventy-five hypermethylated CpG sites linked to 164 genes, including *CCS* and *DUSP2*; and 306 hypomethylated CpG sites linked to 144 genes, including

GSTM1, *NRGN*, *IL32,* and *CD226* in naïve $CD4^+$ T cells, were identified in African Americans compared to European Americans. A substantial hypomethylation in *CD226* in African American naïve $CD4^+$ T cells was identified. The gene is expressed on the surface of natural killer T cells, plays a vital role in activation, adhesion, cytotoxicity, and differentiation of T cells, and is a genetic risk locus for SLE (Coit et al. 2015; Lofgren et al. 2010). The genetic risk locus was situated downstream of the *CD226* gene body while the hypomethylated CpG site is within 1500 bp of the transcription start site. The study found that several differences in site-specific methylation between ethnicities could partially be attributed to genetic variants [SNP rs55661361 (A/G)] which influence methylation levels of CpG sites. The 30 most hypomethylated CpG sites included four SNPs (substitutions or insertion–deletions), three of which were associated with genes including *BPIFA3*, *NRGN,* and *INTS1*. The association between DNA methylation and genetic/epigenetic variants are elaborated in the "Association of DNA methylation with genetic and other epigenetic variants in various populations" section. The same genes were also found to be hypomethylated in lupus patients. CpG sites, such as cg18938907, cg11680055, and cg24506221 of the *GSTM1* gene; cg00471190 and cg00239353 in the promoter region of the *IL32* gene were hypomethylated in African Americans compared to European Americans. The *GSTM1* gene is involved in the metabolism of products of oxidative stress and xenobiotic compounds including carcinogens; and *IL32* is a cytokine that induces the production of *IL8, MIP-2,* and *TNF* in monocytic cell lines. In addition, *IL32* expression in activated $CD4^+$ T cells was found to be concomitant with increased apoptosis and activation-induced cell death (Coit et al. 2013; Kim et al. 2005).

In South African research, two recent medical studies have found differential DNA methylation in Blacks (Matatiele et al. 2015) and Coloreds (Masemola et al. 2015). Matatiele et al. (2015) found certain genes, such as *PRF1* and *ITGAL* which were consistently hypomethylated in patients of SLE and Systemic Sclerosis, and *CD70* and *CDNK2A* which were consistently hypermethylated in both diseases. Masemola et al. (2015) studied foetal alcohol syndrome (FAS) in Coloreds from Western and Northern Cape of South Africa by analysing four imprinted control regions (*H19 ICR, IG-DMR, KvDMR1* and *PEG3* DMR). The study found significant differences in methylation levels of patients at the two maternally imprinted loci, *KvDMR1* and *PEG3 DMR*, which showed lower average locus-wide methylation in the FAS cases. The authors found that for *PEG3* DMR, female patients/cases had an estimated 1.11% lower methylation than males and in contrast, for the controls, males had a significant estimated 0.84% lower methylation compared to females. For all other regions (*H19 ICR, IG-DMR,* and *KvDMR1*), methylation did not differ by sex in patients, nor in the controls. Age was found to influence DNA methylation in patients; in eight out of ten *IG-DMR* sites, one *KvDMR1* site and one *PEG3* DMR site with a decrease in methylation between 0.11 and 0.43% for every additional year. However, while these studies have researched differential DNA methylation in South Africa, they have not studied it across various ethnic groups, which merit further research for therapeutic intervention.

Loss of imprinting or hypomethylation and loss of imprinting at *KvDMR1* have been widely implicated in Beckwith–Wiedemann syndrome, which is a congenital disorder characterized by pre-and postnatal overgrowth, organomegaly, and a high possibility of childhood tumours (Gaston et al. 2001). The significant loss of DNA methylation (~7%) of the *PEG3 DMR* leads to overexpression, which is implicated in intrauterine growth restriction (Ishida and Moore 2013). Variation of DNA methylation patterns associated with diseases in different human populations is summarised in Table 1.

Non-disease-associated variations in DNA methylation patterns between populations

There is mixed evidence for variations in DNA methylation between African Americans and Caucasians; however, a predominantly lower level of genomic DNA methylation in subjects of African American descent has been reported. To ascertain whether variation in DNA methylation patterns between ethnic groups was also observed at birth, Adkins et al. (2011) examined genomic DNA from umbilical cord blood from healthy newborn Africans and Caucasians. Sixty-eight percent of CpG sites examined (of 3623 CpG sites) showed significantly lower methylation levels in African newborns. This was in complete correspondence with previous reports by Terry et al. (2008), who found that adult Africans/Blacks were found to display lower levels of methylation than adult Caucasians/Whites and Hispanics even after factors, such as prenatal smoke exposure, birth length and weight, smoking, passive smoking and family socio-economic status, were taken into consideration; and Zhang et al. (2011b) who studied *LINE1* (long interspersed nuclear element) of peripheral blood from Hispanics, Blacks and Whites also found lowest methylation levels of 73.1% in Blacks, whereas methylation of Hispanics and Whites were 74 and 75.3%, respectively (Kader and Ghai 2015; Terry et al. 2008; Zhang et al. 2011b). Similar to Terry et al. (2008), Zhang et al. (2011b) found that there were no observed differences in methylation when adjusting for variables, such as smoking, alcohol, body mass index, and education (Zhang et al. 2011b).

While many research studies demonstrating reduced methylation in healthy tissues and genes of Blacks (Adkins

et al. 2011; Terry et al. 2008; Zhang et al. 2011b) may provide significant contributions to long-term disease studies and human identification in forensics, this is not a general trend. Figueiredo et al. (2009) used pyrosequencing to determine global methylation levels of *LINE1* from colonic tissue. Their research found that, in addition to *LINE1* methylation levels in normal mucosa from the left bowel being significantly higher than those on the right side, there were similar levels of methylation in samples from African Americans and Caucasians, whereas Hispanics displayed higher levels of methylation. The authors admitted limitations of their report being; use of a convenience sample, limited variability in methylation values and restrictions of their pyrosequencing assay. However, these findings must be taken into account as similar methylation levels were identified in African Americans and Caucasians. Early reports have indicated that Black infants have been shown to have lower birth weights than their White counterparts in the United States (Kuzawa and Sweet 2009; Martin et al. 2005), and hence, Straughen et al. (2015) examined *Igf1* (insulin-like growth factor 1, essential in placental and foetal growth) of the mononuclear fraction of umbilical cord blood to model the role of methylation as a mediator between birth weight and race. This informative study revealed that higher methylation levels of *Igf1* in Black neonates partly accounted for variation of expression, thus resulting in a substantial decrease in birth weight when compared to non-Black counterparts. While the group acknowledged that *Igf1* is complex in structure due to presence of two promoters and several transcription start sites, and hence, specific inferences concerning gene expression were problematic, and they hypothesised that differences in DNA methylation between races may be related to variation in gene expression. This reciprocally influences birth weight and, eventually, increased risk of disease including diabetes, high blood pressure, abnormal cholesterol profiles and elevated risk of coronary vascular disease across the life course (Kuzawa and Sweet 2009; Straughen et al. 2015).

Based on a small sample size of healthy Hispanics, Blacks and Whites within the ages of 45 and 75, Zhang et al. (2011b) also reported lowest methylation in Blacks, upon analysis of *LINE1* in peripheral blood; however, an age-range wider than between 45–75 years could have provided more significant results. In analysis of skin samples from Africans, Whites and Asians, Winnefeld et al. (2012) found that the intragenic CpG island of the *CPXM2* gene (carboxypeptidase X member II) was only 8% methylated in Asians, but 32 and 38% hypermethylated in Whites and Africans, respectively; the promoter and 5′ region of the *PM20D1* peptidase gene was 39% hypermethylated in Whites and hypomethylated in the others; 9 and 6% methylation in Asians and Africans, respectively. And finally, the 5′ gene body of the *VWCE* (von Willebrand factor C and EGF domains) gene was hypermethylated, exhibiting 61% in Africans, but lower levels of 36 and 37% methylation in Whites and Asians, respectively. The authors did not test the underlying factors that contributed to the varying methylation patterns from samples obtained from differing ethnic groups; however, it was noted that ethnic methylation differences can possibly be explained by the involvement of chromatin factors and/or genetic variations (Winnefeld et al. 2012).

A comprehensive study by Song et al. (2015) discovered ethnic differences in DNA methylation in normal breast tissues obtained from European American and African American women. Following the removal of 4242 probes with detection of $p \geq 0.05$, 11,650 sex chromosome-linked probes, 115,996 probes including SNPs, or within 10 bp of the target CpGs (145,116) and problematic non-specific probes to remove potential bias, a total of 485 ethnic-associated differentially methylated CpG sites were identified, 282 of which were hypermethylated in Europeans and the remaining 203 hypermethylated in African Americans. Differential DNA hypermethylation in promoters occurred nearly half as frequently in African American women than in the European women; hypermethylated differentially methylated CpGs in African Americans were localised mostly to the gene body. The researchers found that the differentially methylated CpGs were enriched in genes associated with cancer, partaking in cell survival and death and cell-to-cell signalling. These genes included the oncogene *TNK2* and tumour suppressors (*AHRR, PACS2,* and *OPCML*). When analysing DNA methylation and gene expression, two genes, *GPX1* and *PLA2G4C,* were also significantly differentially expressed between the European Americans and African Americans at $p < 0.05$. This study highlights that not only do ethnic-associated variances in DNA methylation occur in diseased breast tissues, but also in healthy tissues hence providing further understanding into the role of epigenetic mechanisms in carcinogenesis for European American and African American women. Non-disease associated variation of DNA methylation patterns in different human populations is summarised in Table 2.

Association of DNA methylation with genetic and other epigenetic variants in various populations

Ample evidence for the genetic control of DNA methylation has arisen from association between DNA methylation, histone modifications (Bell et al. 2011; Moen et al. 2013) and single-nucleotide polymorphisms (SNPs) located at specific CpG sites. SNP allele frequencies are known to differ considerably among populations with varying geographic ancestries suggesting that ethnic differences in

DNA methylation could likely be due to differences in population specific alleles or haplotypes that shape and influence CpG and global methylation levels (Altshuler et al. 2010; Cappetta et al. 2015; Fagny et al. 2015). Numerous SNPs serving as quantitative trait loci and affecting DNA methylation (mQTLs) and gene expression (meQTLs) in certain tissues have been identified. Moreover, a single SNP often affects the fate of more than one nearby CpG sites methylation (Adkins et al. 2011; Bell et al. 2011; Gibbs et al. 2010; Heyn et al. 2013; Mendizabal et al. 2014; Moen et al. 2013).

Bell et al. (2011) analysed methylation levels of 22,290 CpG sites in the promoter regions of 13,236 genes of 77 human lymphoblastoid cell lines (LCLs) from the HapMap Yoruba Collection, using the Illumina HumanMethylation27 DNA Analysis BeadChip Assay. Yorubans are an African ethnic group, chiefly of Nigerian descent. These researchers identified 180 CpG sites in 173 genes that were associated with nearby SNPs which were located within 5 kb and in *cis,* and 37 CpG sites associated with SNPs located in *trans* at a false discovery rate (FDR) of 10%. Upon examination of distal associations at SNPs that were implicated in methylation, they detected a significant association between SNP rs8075575 (C/T), and methylation at cg24181591 in gene *EIF5A* (encodes a translation initiation factor); and another strong *cis* association between SNP rs2187102 (A/G) with cg27519424 in gene *HLCS*, which is thought to be involved in mediating histone biotinylation. This study has only identified these mQTLs in single populations without comparisons to others, warranting further research to determine if they differ and hence may be of medical or forensic relevance.

Bell et al. (2011) found that methylation and histone modifications are not mutually exclusive in regulation of gene expression; a resilient negative correlation was detected between histones that target expressed genes and methylation levels, such as H3K9ac, H3K27ac and H3K4me3. These particular histone marks, along with a few others were observed to exhibit lower levels of differential methylation in HapMap Yoruban LCL samples when compared to those of European origin by Moen et al. (2013). Moen et al. (2013) profiled differential methylation in LCLs between the Yoruban and Caucasian residents of European ancestry from Utah (CEU), by Illumina 450 Array. Prior to DNA methylation analysis, 141,763 probes (from a total of 482,421 probe sequences) were removed, which could potentially cross-hybridize, and probes which contained common SNPs within 20 bp of the interrogated CpG sites that had minor allele frequency of less than 0.01 (from dbSNP v135) resulting in final analysis of 283,540 autosomal CpG probes in 73 Yoruban samples and 60 European samples. Significant population-specific differences (13% of differential CpG sites) were observed in analysed CpGs. European samples exhibited higher differential methylation in the regions 1-kb upstream of transcription start sites to the first gene body quantile, with fairly depleted regions towards the 3′ UTRs, whereas the opposite was true for the Yoruban population who exhibited elevated differential methylation more towards the 3′UTRs. A negative correlation between promoter methylation of cg27270541 and expression levels was eminent for the *PLA2G4C* gene (Phospholipase A2, group IVC) in the European population, contrasting with high expression levels when elevated methylation was detected in the gene body (Moen et al. 2013). When testing SNP associations across populations, Moen et al. (2013) selected SNPs that had genotypes in at least 96 samples with minor allele frequency of greater than 0.05 across both populations, and that were not significantly deviated from the Hardy–Weinberg equilibrium ($p = 0.0001$). For high detection power, only those mQTLs within 100 kb of the target CpG sites were focused on. This enabled detection of a shared mQTL between the Yorubans and Europeans; the *G* allele of rs2776937 which was concomitant with lower methylation level of cg10312802 located in the *NRP1* gene (Neurophilin 1), and a population-specific mQTL noted in this study was the *G* allele of rs28544087 (A/G); associated with higher methylation level of cg09307883 in *ANAPC2* (anaphase promoting complex subunit II) in the Yoruban, but not European LCLs. A total of 17,643 mSNPs were associated with 1918 CpGs were found in the Yoruban samples, and 23,924 mSNPs associated with 1354 CpGs were found in the European samples (Moen et al. 2013).

The investigation by Moen et al. (2013) accentuated the concept of methylation playing a major role in gene expression. The group used RT–PCR was to measure the expression levels of several genes and observed that *T* allele of meQTL rs10779587 (A/T) exhibited high methylation levels of cg01313622, concomitant with reduced expression of *FLVCR1* (feline leukaemia virus subgroup C cellular receptor I). The *T* allele was less frequent in the Yoruban samples, and thus, higher expression levels were obtained. A sizable number of mQTLs were linked to racial differences in neurological, metabolic, and autoimmune disorders. For example, three SNPs associated with rheumatoid arthritis, multiple sclerosis, and prostate cancer were annotated as a mQTL for a CpG located intergenically within *LOC285830* (homo sapiens hypothetical LOC285830). All three SNPs demonstrated a higher risk allele frequency in the European LCL samples as opposed to Yoruban. In addition, five SNPs associated with cardiovascular diseases and cholesterol levels were annotated as mQTLs for cg12556569 in the promoter region of *APOA5* (apolipoprotein A–V). These sites had higher methylation levels in Yoruban samples and thus posed a higher risk allele frequency.

Another study based on lymphoblastoid cell lines (LCLs) from the HapMap project between 30 family trios (mother/father/offspring) of European and Yoruban populations by Fraser et al. (2012), detected several of differentially methylated CpG sites within, as well as between populations using the Infinium HumanMethylation27 BeadChip assay. The group investigated the methylation status of 27,578 CpG sites near transcription start sites of 14,495 genes. Over a third of the genes examined differed between the populations. DNA methylation was found to be liable for little differential gene expression that existed between the groups. Measuring the link in methylation levels between parents and offspring revealed transmissible methylation at about 900 CpG sites in the Yoruban population, and over 700 in the European LCLs were discovered. This was implicative of frequent genetic control of methylated polymorphisms. Divergences impacting heritability of methylation were further investigated by comparing the SNPs linked to methylation of CpG sites (mSNPs) in the populations, as genetic and environmental interactions may have led to reduced heritability. A requirement for the mSNP analysis was a minimum of 5 minor alleles among the 90 individuals of each population to include an SNP. After the removal of probes which could lead to technical variation in results (2734 probes in Europeans; 3923 probes in Yorubans), 86 and 49 local mSNPs (those within 100 kb of the CpG site) were identified in the Yoruban and European groups, respectively, which accounted for 36–92% observed differential methylation. Neither population's mSNPs affected DNA methylation in imprinted regions. There was a minimal overlap in the mSNPs from the populations, i.e., only 8.9% of the mSNP sites overlapped; and hence, inconsistencies in allele frequencies of SNPs between the populations may have led to lower heritability. Upon exploring this reasons for lack of overlap between the mSNPs, Fraser et al. (2012) found that several mSNPs exerted population-specific effects on DNA methylation which could be accounted for by two factors; genetic interaction with the environment or other variants. For example, the diets between the populations differ, and therefore, some genetic variants have a differing observable effect on methylation only in the presence of methyl donors (Fraser et al. 2012).

Heyn et al. (2013) analysed naive blood and lymphoblastoid cell lines (LCLs) from healthy African Americans, Caucasian Americans, and Han-Chinese Americans using Infinium HumanMethylation450 BeadChip arrays. The group identified 439 CpG sites that showed differential DNA methylation between the three ethnic groups. Out of the 439 CpGs, 178 were located in gene promoter regions, 147 in gene bodies, and 114 in intergenic regions.

The authors found that promoters of genes that harboured the population-specific CpGs (pop-CpGs) were associated with natural human variation between the three populations. Specifically 129, 172, and 138 CpG sites displayed differential DNA methylation patterns in Caucasians, African Americans, and Han-Chinese Americans, respectively. The pop-CpGs were located in genes linked to xenobiotic metabolism and transport (examples *GSTT1* and *SPATC1L*), immune response factors (*CERK, CD226, LCK, SEPT8*) and environmental information processing and adaptation (*ARNTL, PRSS3*, *CNR2*). Some pop-CpGs were also linked to genes that contribute to diseases among the populations, such as onset of Parkinson's disease (*PM20D1*), HIV infection (*HIVEP3, HTATIP2, CDK11B*), and measles virus infection (*FYN*). Nearly 300 of 439 of differentially methylated CpG sites were significantly related to underlying genetic variation (596 SNPs, random forest selection frequencies, FDR <0.05). meQTLs were located close to the correlated SNP site, with 412 out of 596 present within a 15-kb window and 227 out of 596 within 5 kb. Seventy-nine out of the 596 SNPs related to differentially methylated pop-CpGs were located in exonic regions. An example of the connection between ethnicity-associated epigenetic and genetic marks was *SPATC1L*; the gene was previously identified as a CpG methylation quantitative trait loci (meQTL) and expression quantitative trait loci (eQTL) in a screening of Yoruba individuals by Bell et al. (2011). The entire promoter region of *SPATC1L* was differentially methylated in African Americans with high correlation between genotype and gene expression. Six SNPs were directly related to CpG sites that were differentially methylated between populations; the SNPs were located within the *HLA-DPA1* and *HLA-DPB1* locus, and were strongly associated with chronic hepatitis B virus (HBV) infection. These were more abundant in those of Asian and African ancestry. DNA hypermethylation of the *HLA-DPA* promoter led to gene repression, and thus, the group identified risk alleles for mediating DNA methylation variation and gene repression, which, in turn, gives rise to altered cell surface receptor presentation and thus altered HBV binding and infection risk. These distinct patterns of differential DNA methylation enabled the authors to associate the populations to define phenotypic characteristics, such as drug metabolism, disease susceptibility, response to environmental stimuli and sensory perception.

As previously mentioned, allele frequencies among SNPs can vary considerably among populations with different geographic ancestries (Altshuler et al. 2010; Cappetta et al. 2015). Adkins et al. (2011) examined African Americans and Caucasians, and Gibbs et al. (2010) and Zhang et al. (2010) examined Caucasians only; however, a similar proportion of CpGs associated with SNPs were found in all three studies (4–8.6%) suggesting that variation in SNPs allele frequencies of African American and Caucasian cohorts may not fully account for ethnic differences

between them. In contrast, Heyn et al. (2013) who studied LCLs and blood from African Americans, Caucasian Americans and Han-Chinese Americans, found that 68% (298 of 439) of differentially methylated CpG sites were significantly related to underlying genetic variation. Toperoff et al. (2012) who researched diabetes in Ashkenazi Jews found that *A* allele of rs1121980 (an established risk factor for obesity and diabetes) near the *FTO* gene in diabetes patients had a frequency of 48.2% in patients and a 43.6% frequency in healthy controls but had no effect on observed methylation differences between patients and controls. Perhaps, only a fraction of racial differences in DNA methylation that studies have reported could be due to variation in the frequencies of SNP alleles or haplotypes between the races.

Modern humans are said to originate in Africa over 200,000 years ago, and then progressively spread across the globe within the past 100,000 years. Ample efforts have been made to characterize genetic variation and relationships between populations of the African continent. Africa is said to be important due to complexity of ethnic populations, variation of diets and climates as well as exposure to disease. All of these factors result in high levels of diversity in genotypic and phenotypic characteristics (Bryc et al. 2010; Campbell and Tishkoff 2008; Tishkoff et al. 2010). In a broad study considering this diversity, Fagny et al. (2015) evaluated DNA methylation profiles of whole-blood samples using the Illumina 450k array, obtained from rainforest hunter-gatherers, also known as Pygmies (w-RHGs), and two populations that adopted Agrarian lifestyles; one that occupied urban deforested habitats (w-AGRs) and another from a forested region of Cameroon (f-AGRs). To compare the methylation profiles of those from the western regions, they also analysed two populations from the Uganda, in the east, namely, e-RHGs and e-AGRs. After normalization and filtering which involved the removal of probes that contained genetic variants at a frequency higher than 1% in the populations studied, a total of 365,886 probes in 352 individuals were assessed. Over 25,000 differentially methylated regions (DMRs) across 8803 genes were identified between the w-RHGs and w-AGRs from the west, and just over 19,000 DMRs across 6288 genes were found between the two eastern populations, whereas nearly 7000 sites within 2528 genes were shared between western and eastern populations. When focusing on SNPs located in *cis* within a 200-kb window around the target CpG sites to search for meQTLs, Fagny et al. (2015) found nearly 46,000 DNA methylation sites that were associated with a nearby meQTL. Interestingly, only 1283 meQTLs were exclusive to the RHG group, and 500 meQTLs were exclusive to the AGR group, whereas majority of these meQTLs overlapped across populations. Such extensive sharing of meQTLs reflects the closer genetic proximity of the populations studied. Common mSNPs that occurred at varying allele frequencies between the populations were found in genes, such as *HOX6C*, *IGFBP2,* and *ZNF492*. These were associated with age of puberty, bone-mineral density and height. These results are consistent with other findings, since several studies have found common loci that correlate with height; the *CISH-MAPKAPK3-DOCK3* region located in chromosome 3 being one of them (Jarvis et al. 2012; Lachance et al. 2012; Perry et al. 2014). *CISH, MAPKAPK3,* and *DOCK3* were all differentially methylated between the populations (Fagny et al. 2015). Genetic variation in *DOCK3* has also been linked to height in a study of Europeans by Wood et al. (2014); and variations in *CISH* influence the human growth pathway and have been linked to increased susceptibility to diseases, such as bacteraemia, malaria, and tuberculosis in African populations (Khor et al. 2010).

In the study based on methylation differences in African-American and European-American SLE patients by Coit et al. (2015), *cis*-acting genetic variants were found to be responsible for some population-specific methylation differences. The Illumina Infinium HumanMethylation450 microarray was used to study differential methylation between African-American and European-American individuals on 425,161 probes. To avoid probe hybridization and technical errors, the researchers determined the presence of SNPs with a minor allele frequency of >1% located within the top 30 hypomethylated CpG dinucleotides in African Americans. The 30 CpG sites included four SNPs (substitutions or insertion–deletions), three of which were associated with genes including *BPIFA3* (*C20orf71*), *INTS1,* and *NRGN*. In the site that was most hypomethylated in the African-American group in the *NRGN* gene, an intragenic SNP was located (i.e. rs55661361). The *A* allele alters the CpG site to a CpA site, hence making methylation impossible at this locus. This *A* allele has a frequency of 79 and 37% in Yorubans and European populations, respectively, which indicated that loss of DNA methylation in this CpG site in African Americans might be explained by this polymorphism. On the other hand, an 803-bp region of the cytokine gene *IL32* (immediately upstream and including the transcription start site and its 5′-untranslated region) contained four CpG sites (mentioned previously) that were hypomethylated in African Americans; this methylation level was found to be independent of any SNPs.

Methylation differences in over 500 Puerto Rican and Mexicans were recently assessed by Galanter et al. (2016). Primary blood was collected and, using Infinium HumanMethylation450 BeadChip arrays, the researchers found 916 CpG differentially methylated sites between the two ethnicities. These results were found after probes on sex chromosomes were excluded, and as well as those containing genetic polymorphisms in the probe sequences.

Table 1 Summary of disease-associated variations of DNA methylation patterns between populations

Disease/illness	Target gene	Region of gene/site of interest	Hypomethylation (*n* participants)	Hypermethylation (*n* participants)	References
Malignant mesothelioma	IGTBP-3	Promoter	USA (40)	Japan (16)	Tomii et al. (2006)
Head and neck squamous cell carcinoma	LRE1	160 bp region; i.e. restriction products of *Tas*I (63 and 97 bp)	Boston Caucasians (258)	Boston non-Caucasians (20)	Hsiung et al. (2007)
Prostate cancer	*GSTP1*	Promoter	Caucasians (77) Asians (170)	African Americans (44)	Enokida et al. (2005)
Prostate cancer	*GSTP1, AR, RARβ2, SPARC, TIMP3, NKX2-5*	Promoters of all stated genes	Caucasians (12–40)	African Americans (40)	Kwabi-Addo et al. (2010)
Colorectal cancer	*ICAM5, GPNMB, CHD5*	Promoters of all stated genes	Iranians (51)	Blacks (51)	Mokarram et al. (2009)
Breast cancer	*HIN-1, Twist, Cyclin D2, RASSF1A*	Promoters of all stated genes	Caucasian women	African American women	Mehrotra et al. (2004)
Diabetes	FTO	CpG site located within intron Chr 16:53809231-2; hg19	Palestinians (929)	Israeli Jews (629)	Toperoff et al. (2015)
Diabetes	*TXNIP*	cg19693031 (Chr 1:145441552)	Europeans (7066)	Indian Asians (13,535)	Chambers et al. (2015)
	ABCG1	cg06500161 (Chr 21:43656587)			
	SOCS3	cg18181703 (Chr17:76354621)			
	PHOSPHO1	cg02650017 (Chr17:47301614)			
	SREBF1	cg11024682 (Chr17:17730094)			
Lupus	*IL32*	cg08978665 (Chr 16:3115707)	African Americans (21 healthy; 21 patients)	European Americans (45 healthy; 42 patients)	Coit et al. (2013)
		cg00471190 (Chr 16:3115809)			
		cg00239353 (Chr 16:3115133)			
		cg23813257 (Chr 16:3115286)			
	CD226	cg20768743 (Chr 18:67624846)			
	CDKN1A	cg24425727 (Chr 6:36645648)			
	GSTM1	cg18938907 (Chr 1:110230456)			
		cg11680055 (Chr 1:110230252)			
		cg24506221 (Chr 1:110230401)			
		cg10950028 (Chr 1:110230633)			
	PTPRN2	cg22216157 (Chr 7:157643037)			

Table 2 Summary of non-disease-associated variations of DNA methylation patterns between populations

Tissue	Target gene/genes associated	Region of gene/site of interest	Hypomethylation (*n* participants)	Hypermethylation (*n* participants)	References
Genomic DNA, umbilical cord blood	–	26485 autosomal CpGs	African new-borns (107)	Caucasian new-borns (94)	Adkins et al. (2011)
Peripheral leukocytes	LINE1	–	African Americans (69)	Caucasians (33) Hispanic (58)	Zhang et al. (2011b)
Colonic tissue	LINE1	–	African Americans (22), Caucasians (325), mixed race (19)	Hispanics (22)	Figueiredo et al. (2009)
Umbilical cord blood	Igf1	Chr 12:101398416	Blacks (21)	Non-Backs (66)	Straughen et al. (2015)
Skin	CPXM2	Intragenic CpG Island	Asians (10)	Caucasians (10), Africans (10)	Winnefeld et al. (2012)
Skin	VWCE	5′ Gene body	Caucasians (10), Asians (10)	Africans (10)	
Skin	PM20D1	Promoter and 5′ gene region	Asians (10), Africans (10)	Caucasians (10)	
Breast tissues	*TNK2, AHRR, PACS2, OPCML*[a]	Promoters of all stated genes	African Americans (22)	Europeans (61)	Song et al. (2015)
Umbilical cord blood	*PIK3CA, NRAS, APC, MCC*[a]	15,280 promoter CGIs and 5315 non-CGIs outside promoter regions	African Americans (112)	European American (91)	Mozhui et al. (2015)
LCLs	*STK39*	Regions 1 kb upstream of transcription start site	Yoruban (74) Europeans (60)		Moen et al. (2013)
	–	Histones associated with methylation; H3K9ac, H3K27ac and H3K4me3			
Whole blood	–	cg19145607	Puerto Ricans (220)	Mexicans (276)	Galanter et al. (2016)

Zhang et al. (2011b) and Figueiredo et al. (2009) measured *LINE1* methylation as an indicator of global methylation

[a] Several more listed in article

A considerable association with ethnicity was observed at cg12321355 in the ABO blood group gene *(ABO)* on chromosome 3 with p value 6.7×10^{-22}. Upon stratification for ethnic subgroup, an association in both Mexicans ($p = 0.003$) and Puerto Ricans ($p = 0.001$) was observed. One locus in particular cg19145607 was found to be hypomethylated in Puerto Ricans but hypermethylated in Mexicans ($p = 1.4 \times 10^{-19}$). In addition to finding an association between environmental factors and methylation (Table 3), upon examination of association between methylation levels at each CpG site and ancestry, out of 361,943 CpG sites, the study identified 3694 sites that were linked to ancestry. Most noteworthy associations were found at cg04922029 and cg06957310 on chromosome 17. The study participants were genotyped at 818,154 SNPs and those SNPs with >5% missing data were removed, along with those out of Hardy–Weinberg equilibrium and non-autosomal SNPs. Methylation of 98% of the loci in the admixture mapping findings was linked to SNPs, with the furthest distance between SNP and CpG site being 998 kb apart. Methylation at cg25134647 correlated with rs4963867 (C/T) on chromosome 12, and cg04922029, which showed significant admixture association, was correlated with rs2814778 (A/G). This latter association, situated on the *DARC* gene confers resistance to *P. vivax* malaria and is referred to as the Duffy null mutation, is ubiquitous with 100% frequency in the five 1000 genomes populations of Africa (1000 Genomes Project Consortium 2010, 2012), including Western Gambians, Esan in Nigeria, Luhya in Webuye, Kenya, and Yorubans of Nigeria; 89 and 80% in Afro Caribbeans in Barbados and African Americans in the Southwest US, respectively; the Latinos studied by Galanter et al. (2016) had minor allele frequencies of 14% in Puerto Ricans and 3% in Mexicans.

When drawing conclusions on the association of methylation and phenotype, it is essential to note that the presence of genetic variants such as SNPs affects the regulation of methylation, and the ensuing expression of a phenotype.

Table 3 External factors shown to alter DNA methylation levels in various populations

Factor/s	Tissue	Gene	Region of gene/site of interest	Population/s studied (*n* participants)	Population age groups (years)	References
Age	Lymphocytes	–	–	Icelandic cohort (111); Utah population (680)	Icelandic cohort 70–82 years; Utah population 5–72 years	Bjornsson et al. (2008)
Age	Peripheral leukocytes	LINE1	–	African Americans (69); Whites (33); Hispanics (58)	45–75	Zhang et al. (2011b)
Age	Peripheral venous blood	*GPR137*	11q22.3	Chinese population (105)	10–72	Yi et al. (2014)
		MEIS1	2p14			
		UBQLN1	9q22			
		TBOX3	12q24.1			
		ZIC4	3q24			
		UBE2E1	3p24.2			
		PTDSS2	11p15.5			
		ZDHHC22	14q24.3			
Age	Whole blood, cord blood	*ELOVL2*	CGI; cg16867567	Italians (64)	Mothers 42–83 years; offspring 9–52 years	Garagnani et al. (2012)
			CGI; cg 21572722			
			CGI; cg24724428			
		FHL2	CGI; cg06639320			
			CGI; cg22454769			
			CGI; cg24079702			
		PENK	CGI; cg16419235			
			CGI; cg16219603			
			CGI; cg12877723			
Age	Blood	TRIM45	cg04400972 (Chr 1:117665053)	Caucasians (426); Hispanics (230)	19–101	Hannum et al. (2013)
		SFMBT1	cg03607117 (Chr 3:53080440)			
		POU4F2	cg02650266 (Chr 4:147558239)			
		PENK	cg16419235 (Chr 8:57360613)			
		TEAD1[a]	cg04940570 (Chr 11: 12696758)			
Age	Whole blood leukocytes	ELOVL2	cg16867657	Germans (965)	50–75	Florath et al. (2014)
		FHL2	cg06639320			
		ZYG11A	cg06784991			
		NEFM[a]	cg07502389			

Table 3 continued

Factor/s	Tissue	Gene	Region of gene/site of interest	Population/s studied (*n* participants)	Population age groups (years)	References
Age	Peripheral venous blood	ELOVL2	cg21572722 (Chr 6:11044661) cg24724428 (Chr 6:11044655) Chr 6:11044647 cg16867657 (Chr 6:11044644) Chr 6:11044642 Chr 6:11044640 Chr6:11044634	Polish population (427)	2–75	Zbiec-Piekarska et al. (2015)
Dietary folate	Leukocytes global methylation	*LINE1*	–	New York population (165)	18–78	Zhang et al. (2012)
Dietary folate	Lymphocytes			Californians (8)	Post-menopausal women 49–63	Jacob et al. (1998)
Dietary folate	Leukocytes			Gainesville (Florida) (33 years)	Elderly women 60–85	Rampersaud et al. (2000)
Dietary folate	Peripheral blood mononuclear cells			Italians (292)	Varied ages	Friso et al. (2002)
Dietary folate	Leukocytes			Mexican-American women (43)	18–45	Axume et al. (2007a)
Dietary folate	Leukocytes			Caucasians (41)	20–30	Shelnutt et al. (2004)
Vitamin D	Neonatal cord blood	*PIK3CA, NRAS, APC, MCC*[a]	15280 promoter CGIs and 5315 non-CGIs outside promoter regions	European Americans (91) African Americans (112)	16–40 years; 16–28 weeks of gestation	Mozhui et al. (2015)
Heroin	Peripheral blood lymphocytes	OPRM1	Promoter	Caucasians (329); Hispanics (203); African Americans (198)	Caucasians varied ages; Hispanics 18–60 years African Americans 19–70 years	Nielsen et al. (2009, 2010)
Smoking	485577 CpG sites in whole blood: monocytes, granulocytes, NK cells, B cells, $CD8^+$-T cells and $CD4^+$-T cells	AHRR	cg05575921 cg14817490 cg21161138 cg25648203 cg21161138	Arabs (123)	72 females mean age 39 years; 51 males mean age 36.3 years	Zaghlool et al. (2015)

Table 3 continued

Factor/s	Tissue	Gene	Region of gene/site of interest	Population/s studied (*n* participants)	Population age groups (years)	References
Smoking	Cord blood	AHRR	cg05575921	Norwegians (1062)	Newborns; mothers mean age 29 years	Joubert et al. (2012)
			cg21161138			
			cg25648203			
		GFI1	cg09935388			
			cg09662411			
		MYOIG	cg22132788			
			cg04180046			
		CNTNAP2	cg25949550			
Smoking	Whole blood	AHRR	cg05575921	Germans (1793)	32–81	Zeilinger et al. (2013)
			cg21161138			
			cg25648203			
		GFI1	cg09935388			
			cg09662411			
		MYOIG	cg22132788			
			cg04180046			
		CNTNAP2	cg25949550			
		TMEM51	cg09069072			
Smoking	Lymphoblast and lung alveolar macrophage	*AHRR*	cg14817490	Caucasians (165)	43–47	Monick et al. (2012)
Smoking	Peripheral blood	*AHRR*	cg05575921 (Chr 5:373378)	European and South Asian males (192)	40–69	Elliott et al. (2014)
		6p21.33	cg06126421 (Chr 6:30720080)			
		GFI1	cg09935388 (Chr 1:92947588)			
		F2RL3	cg03636183 (Chr 19:17000585)			
		TMEM51	cg09069072 (Chr 1:15482753)			
		GNG12	cg25189904 (Chr 1:68299493)			
Smoking	Whole blood	AHRR	cg23067299	Puerto Ricans (220); Mexicans (276)	8–21	Galanter et al. (2016)
Diesel exhaust particles		RPS27A	cg05084827			
		PHTF2	cg27457191			
		CASP7	cg07462448			
		ZNF121	cg08131547			
Exposure to violence		ADCYAP1R1	cg11218385			

[a] Several more listed in article

Several studies using the Illumina array-based method have also acknowledged that SNPs within probes themselves can affect probe binding and, hence, lead to technical artefacts. Therefore, as mentioned in studies above, to eradicate these issues researchers eliminate CpGs based on the SNP annotations of the Illumina manifest. Moreover, in some cases to remove potential bias between males and females, researchers sometimes remove the X and Y chromosome probes (Zaghlool et al. 2015).

In addition, many of the studies mentioned in this review have detected differences in DNA methylation levels between populations by use of LCLs (Bell et al. 2011; Heyn et al. 2013; Fraser et al. 2012; Moen et al. 2013). LCLs are generally the most feasible, convenient approach to conduct population-based studies in humans, since other samples are extremely difficult to collect and maintain. Methylation analysis using LCLs between populations has the advantage of being a pure population of B cells. Primary samples from humans can include more than one cell type, which may show differential methylation. This can be erroneously thought to be as population specific differential methylation (Moen et al. 2013). Cell lines offer replicability; and particularly, the HapMap LCLs represents the most complete catalogue of human variation and is thus frequently used as a model system for functional studies based on humans. However, there is now a lingering concern around the use of LCLs to infer population-based DNA methylation variation (Caliskan et al. 2011; Grafodatskaya et al. 2010; Sun et al. 2010). Studies investigating differences in methylation levels between LCLs and peripheral blood cells have found significant discrepancies in methylation profiles of the cell types and subsequent gene expression. Such studies have shown that EBV transformation and short-term culture may induce random DNA methylation alterations, and extended cell culturing and repeated freeze–thaw cycles encourage progressive random changes in DNA methylation. On the other hand, some studies, such as Fraser et al. (2012), have validated their results obtained with the use of LCLs; as their samples were processed randomly, thus eliminating the likelihood of batch effects that influenced estimates of population specificity; and cell line artefacts were eliminated as a causative factor of observed variation since majority of the population-specific DNA methylation was explained by the presence of local genetic variants. Additional validation for population-specific mSNPs found by Fraser et al. (2012) was that similar results for Europeans were found by Gibbs et al. (2010) and Zhang et al. (2008). Similarly, Bell et al. (2011) and Moen et al. (2013) assessed confounding factors for LCLs such as intrinsic growth rate and EBV copy number and found that their results too were not influenced by such factors. Nevertheless, it is still essential to take these factors into consideration and exercise caution when inferring population variation based on methylation levels of LCLs. This definitely warrants further investigation of another tissue or cell type to detect DNA methylation variation between populations, especially during identification of disease-associated DNA methylation changes or for discovery of new imprinted genes (Caliskan et al. 2011; Grafodatskaya et al. 2010; Sun et al. 2010).

Environmental factors contributing to DNA methylation differences among human populations

Research over the past few years has established that individual genetic background and environmental factors are intertwined to lifestyle in determining the overall genetic and hence, health status of individuals. Epigenome-wide association studies have established robust associations of demographic, psychosocial, and environmental factors with DNA methylation patterns (Alegria-Torres et al. 2011; Hunter 2005; Tammen et al. 2013). Factors influencing DNA methylation include, but are certainly not confined to nutrition and diets (King-Batoon et al. 2008; Park et al. 2011), life experiences (McGowan et al. 2009), aging, stress, exposure to pollutants (Alegria-Torres et al. 2011), alcohol (Hines et al. 2001; Mason and Choi 2005) as well as economic status and even institutionalised care (Lam et al. 2012; Naumova et al. 2012). While thorough investigations of the effects of external factors on the DNA methylation status of cells and tissues between populations are still in infancy, researchers have indeed investigated them in single populations.

For example, Lam et al. (2012) assessed external factors that sculpt DNA methylation of peripheral blood mononuclear cells obtained from Caucasians and Asians of Vancouver. The study found that blood composition, age, sex, early life poverty, stress as well as ethnicity lead to variable levels of DNA methylation. The researchers assessed methylation levels of promoters by use of Illumina arrays and found nearly 300 CpG sites (from a total of 17,870) that displayed variable DNA methylation patterns associated with ethnicity. Twenty-one of these 300 CpG sites exhibited more than 5% variation in methylation levels. The study provided informative insights into methylation differences between populations; however, a limiting factor was the failure to assess whether any of the observed variation in methylation was due to the presence of allelic variation at proximate SNPs.

Age

The precise regulation of site-specific age-associated DNA methylation has yet to be established; however, it is well documented that changes in global DNA methylation seem to occur naturally with aging (Calvanese et al. 2009; Day

et al. 2013). Studies have shown that aging cells display a progressive decline in methyl-cytosine (Thompson et al. 2010). Christensen et al. (2009) reported a tendency of CpG sites located outside of CpG islands to lose methylation with progressing age, whereas the opposite was true for CpG islands themselves. Increased DNA methylation contributes to age-related diseases such as neurological disease (Bocklandt et al. 2011; Zaghlool et al. 2015). Bjornsson et al. (2008) obtained an intra-individual global measure of Hpa II/Msp I methylation in participants from Utah and Iceland and found a general genomic decrease in DNA methylation with age; 29% of Icelandic participants showed over 10% variation in methylation over an 11-year period. The Utah population included families, enabling estimation of familial correlations in methylation changes, and this cohort showed similar changes in methylation over a 16-year period.

In a California-based examination of saliva DNA from 34 pairs of twins (21–55 years old), Bocklandt et al. (2011) found 88 CpG sites near 80 genes whose methylation percentage correlated with age; 19 negative correlations, 69 positive correlations. Seventy-three of 88 (83%) significant probes were within CpG islands. Ten of these 88 CpG sites were also correlated with age in whole blood and in isolated $CD4^+$ and $CD14^+$ cells as well (Rakyan et al. 2010).

Florath et al. (2014) studied individuals from Saarland, Germany, and found 162 CpG sites associated with age in blood; 43 of which were negatively correlated. The ten CpG sites most significantly associated with age were mapped to genes *ELOVL2, FHL2, ZYG11A, SLC12A5, OTUD7A* and *CCBC102B.*

Zaghlool et al. (2015) detected variable DNA methylation in 13% of the CpG sites detected by Bocklandt et al. (2011), and in nearly 63% of the CpGs detected by Florath et al. (2014) to be associated with age in an Arab population. This study indicated that a few CpGs associated with aging are similar in European and Non-European populations.

DNA methylation is now widely being used for forensic age estimation, to complement the estimation of phenotypic characteristics and hence provide a more accurate description of unknown individuals. In an effort to search for age-related DNA methylation markers Yi et al. (2014) identified eight novel loci in Chinese individuals that not only show diversity in methylation patterns with age but are also involved in developmental processes. Within the eight loci that were located in eight specific genes, a total of 33 CpGs were found to show obvious association with age; for 51% of which a demethylation was observed. Among the age-related methylation, markers was the *ZIC4* gene located in a CpG island, which codes for a protein which is a member of the ZIC family of zinc finger proteins and is vital during development and disease; and the *UBQLN1* gene, located in promoters and CpG islands, which encodes the ubiquitin-like protein Ubiquilin. It modulates the accumulation of presenilin proteins. However, the study focussed solely on the Chinese population, warranting further research to determine if similarities or differences exist amongst a broader population range.

The DNA methylation status of the *ELOVL2* marker has also been extensively researched for age prediction. The marker was shown to correlate with age in an Italian mother-and-offspring cohort by Garagnani et al. (2012), Caucasians and Hispanics (aged 19–101 years) in the study by Hannum et al. (2013); with 965 Germans in the study by Florath et al. (2014) and 7 age-associated CpG sites within the gene were found in Polish individuals by Zbiec-Piekarska et al. (2015). It would be beneficial to compare the extent/degree of age-related variation in DNA methylation in each of the populations, as well as research it on a broader population range to determine if any population is insusceptible to age-related variations in DNA methylation using the *ELOVL2* marker.

Several researchers are now taking advantage of the mounting evidence of associations of DNA methylation with disease and aging rates to develop epigenetic clocks which capture DNA methylation signatures and a DNA methylation age (DNAm age) with remarkably high correlations with chronological age (Bocklandt et al. 2011; Christiansen et al. 2016; Horvath 2013; Marioni et al. 2015). The DNAm age is understood to be associated with an individual's current and future health and mortality and is, therefore, the subject of much interest. Horvath (2013) developed a multi-tissue predictor of age which allows one to estimate the DNAm age of most tissues and cell types. The 'Horvath Clock' was developed using 7844 samples from 51 healthy tissues and cell types from 82 Illumina DNA methylation array data sets, and 5826 cancer samples from 32 individual cancer data sets. The result was the characterization of 353 CpG sites that together form an aging clock; 160 CpGs correlate negatively with age while the residual 193 of the 353 CpGs correlate positively with age. The clock may be used to measure the DNAm age of human tissues, organs and cell types—including brain, breast, kidney, liver, lung, blood, as well as prenatal brain samples and has been frequently relied upon by many researchers (Levine et al. 2015b; Lu et al. 2015; Marioni et al. 2015). In addition to being highly accurate and applicable to a broad array of tissues and cell types, another salient feature of the Horvath clock is that it was not based on a single population or ethnic group and, therefore, has been used in studies based on Caucasians (Levine et al. 2015a, b), African Americans, Hispanics (Levine et al. 2015a), Europeans (Lu et al. 2015), Danish twins (Christiansen et al. 2016) as well as four large cohorts consisting of Lothian Birth Cohort 1921, Lothian Birth Cohort 1936, the Framingham Heart Study and the

Normative Aging Study (Marioni et al. 2015) and numerous other populations and groups.

Thereafter, other age estimators based on DNA methylation have been developed. Hannum et al. (2013) developed their model using blood but showed that with a calibration step, it is applicable to other tissues, such as breast, kidney and lung tissues. Weidner et al. (2014) described an age estimator for DNA from blood that uses 3 CpG sites, namely, cg02228185 in *ASPA* (aspartoacylase), cg25809905 in *ITGA2B* (integrin, alpha 2b), and cg17861230 in *PDE4C* (phosphodiesterase 4C, cAMP specific). The age estimator by Weidner et al. (2014) applies only to blood. Bekaert et al. (2015) found age-associated CpGs in four genes *ASPA, ELOVL2, PDE4C,* and *EDARADD* that were applicable to blood and teeth; and Giuliani et al. (2015) recently found age-associated CpGs in *PENK, ELOVL2,* and *FHL2* genes for age estimation of teeth.

These epigenetic clocks have tremendous potential, and much work remains to test these clocks and models against various populations. In this way, medical researchers may be able to compare methylation patterns and ages of same tissues from individuals of various ethnic groups and populations, in the hope of identifying less invasive yet accurate methods to diagnose disease or gauge the risk of future illnesses, such as cancers, obesity, Parkinson's and Huntingtons's disease, Alzheimer's, Downs syndrome and even HIV AIDS (Horvath and Levine 2015; Horvath et al. 2014, 2015; Horvath 2015, 2016; Lu et al. 2015). Thereafter, therapeutic intervention is inevitable and may be customized to suit the needs of particular ethnic groups.

Diet

Human studies relating diet and DNA methylation have yielded inconsistent findings. Methyl-donor and methylation cofactor micronutrients play a direct role in DNA methylation pathways. For example, folate is a vital nutritional factor in one-carbon metabolism as it provides the methyl units for DNA methylation (Crider et al. 2012; Mason and Choi 2005). Generally, increased intake of these micronutrients would be concomitant with elevated DNA methylation. However, Zhang et al. (2011a) showed that dietary intake of folate and other one-carbon nutrients was not related to *LINE1* methylation among 149 healthy adults (aged 45–75 years) in Texas, and Perng et al. (2012) studied Colombian school children, and found that neither erythrocyte folate nor serum vitamin B_{12} was associated with *LINE1* methylation.

In contrast, a study of 165 healthy adults, aged 18–78 years, in New York found that increased consumption of folate from fortified foods such as breakfast cereals showed a positive correlation ($p = 0.007$) with methylation levels (Zhang et al. 2012). However, in this study, white blood cell *LINE1* methylation was measured as a surrogate for global DNA methylation by pyrosequencing; hence, the results were not directly comparable to those quantifying global DNA methylation content or assessing other repetitive elements such as *Alu*. Increased folate intake did not significantly affect methylation of the *IL-6* promoter in this study (Zhang et al. 2012).

The key enzyme 5,10-methylenetetrahydrofolate reductase (*MTHFR*) is essential in folate metabolism. The DNA methylation status of the gene may be altered by a common genetic variation in the enzyme; *MTHFR 677C → T* (Axume et al. 2007a, b; Botto and Yang 2000; Friso et al. 2002).

Early reports have shown a higher frequency of the *MTHFR 677C → T* polymorphism in Whites from Italy, Germany, Ireland and Britain as well as Hispanics living in California, and a lower frequency among Blacks from Brazil and US, and in some areas of sub-Saharan Africa (Botto and Yang 2000). One of the common phenotypic outcomes of *MTHFR* variants are neural tube defects, such as spina bifida, anencephaly, and encephalocele. These defects occur, because the neural tube fails to close during foetal development, leading to severe disability or even early death. In particular, spina bifida is associated with bowel disruption, bladder, sexual and motor function. The rate of these neural tube defects was higher in some regions/countries and some ethnic groups; for example, among Chinese from northern China and among Hispanics living in some Latin American countries, and lower in other ethnic groups such as Blacks living in the United States (Botto and Yang 2000). In contrast, some early studies have found minimal association between the *MTHFR 677C → T* polymorphism and neural tube defects (van der Put et al. 1998).

Folate depletion in healthy human volunteers initiates a diminish in genomic DNA methylation in lymphocytes (Jacob et al. 1998; Rampersaud et al. 2000), while Fenech et al. (1998) showed that folate supplementation in a group of young adult Australians had minimal effect on DNA methylation. Friso et al. (2002) evaluated blood from 292 Italians and found that global leukocyte DNA methylation was lower in individuals with the *MTHFR* 677TT genotype compared to the CC genotype, but this was only the case when folate levels were low. Similarly, Axume et al. (2007a) studied a small group of Mexican-American women. The participants consumed a folate restricted diet of 135 μg DFE (Dietary Folate Equivalent)/day for 7 weeks followed by folate treatment with 400 or 800 μg DFE/day for another 7 weeks. After 14 weeks, leukocyte methylation levels were considerably lower in individuals with the *MTHFR* 677 TT genotype than individuals with the 677 CC or CT genotype. In contrast, Shelnutt et al. (2004) assessed global leukocyte DNA methylation in young Caucasian women over a 14-week period; 7 weeks of folate restriction (~115 μg DFE/day) followed by 7 weeks of

folate treatment with 400 μg DFE/day. DNA methylation, as measured by the methyl acceptance assay, increased considerably during folate treatment in women with the MTHFR 677 TT genotype ($p = 0.04$), and dropped significantly during folate treatment in women with the CC genotype (Shelnutt et al. 2004). However, Axume et al. (2007b) explored the effect of race on global leukocyte DNA methylation while controlling folate intake and found that neither race, nor folate intake, was determinants of global leukocyte DNA methylation, since similar methylation levels in 14 African Americans and 14 Caucasians were detected. Controlling dietary folate and/or enzyme activity, and conducting the study using a larger sample size and site/tissue-specific measurement of DNA methylation would be beneficial to determine the exact relationship.

Previous reports have shown that maternal diets also affect the offspring phenotypes and disease-risks (Jimenez-Chillaron et al. 2012; Sakatani et al. 2005; Tammen et al. 2013). A more recent article by Mozhui et al. (2015) examined the influence of maternal diets on the offspring methylome by measuring plasma serum vitamin D and folate during pregnancy. The study covered genome-wide DNA methylation; specifically, 15,280 promoter CpG islands and 5315 non-CpG islands outside of promoter regions, in neonatal cord blood obtained from 91 European American and 112 African American subjects. To avoid errors and hybridization artefacts, the researchers removed 5862 CpG probes that contained an SNP with minor allele frequency greater than 1% in any population and a further 1092 probes that targeted sex chromosomes were eliminated. Significant methylation variation between the two ethnicities were identified at 3802 CpG sites; 2647 CpGs had lower methylation levels in African Americans, whereas only 1155 CpGs were lower in European Americans. About 159 of these differentially methylated CpGs were found to be associated with a nearby meQTL. Similar to Adkins et al. (2011) and Straughen et al. (2015), the researchers found a lower average birth weight in African American newborns (3.18 kg) than in European American newborns (3.52 kg). In addition, both plasma serum vitamin D levels and folate levels were higher in European Americans (20.68 ng/ml vitamin D levels; 32.84 ng/ml folate levels) than in African Americans (17.74 ng/ml vitamin D levels; 28.3 ng/ml folate levels). While both maternal vitamin D levels and folate levels showed limited influences on newborns, vitamin D showed an association with the *TLE1* gene (transducin-like enhancer of split 1) on cg15915418; and folate showed an association with *WDR5* (WD repeat domain 5) on cg03243700. When a weighted correlation method was employed to minimize the data into fewer dimensions and cluster genes having shared variance into tightly correlated networks, race and vitamin D were found to be influential factors. One particular module termed Meth7 comprising genes involved in response to organic substances contained 240 CpG members that showed a correlation with maternal vitamin D levels. In this module, lower vitamin D only among European Americans was associated with higher methylation of CpGs, suggesting a complex relationship of ancestry and vitamin D with DNA methylation. Overall, the study found that maternal vitamin D seems to have a stronger influence than folate on neonate methylomes (Mozhui et al. 2015). The study complemented several other reports that have proven that global methylation is lower in African Americans than in Europeans (Adkins et al. 2011; Straughen et al. 2015; Terry et al. 2008; Zhang et al. 2011b). Several hypomethylated genes in Africans were shown to be enriched in tumour suppressors and cell cycle regulators, as well as colorectal cancer genes (*PIK3CA, NRAS, APC, MCC*). Similarly, Mokarram et al. (2009) found several colorectal cancer genes (*CHD5, GPNMB3, ICAM5*) to be hypomethylated in African Americans when compared to Iranians. This emphasises the role of DNA methylation associated with higher expression of genes involved in cell cycle regulation in colorectal cancer, concomitant with the unequal cancer burden among African Americans.

Drugs and substance abuse

Aberrant DNA methylation has been linked to use of illicit drugs and substance abuse, which may play an essential role in an individuals' inclination towards developing an addiction or the response to pharmacotherapy (Kumar et al. 2005; Nielsen et al. 2009; Novikova et al. 2008). Nielsen et al. (2009) studied methylation at 16 CpG sites (in the upstream CpG island) of the *OPRM1* gene in peripheral lymphocytes from Caucasian former heroin addicts, who were maintained on methadone treatment. The researchers found high methylation levels of two CpG sites in the μ-opioid receptor (*OPRM1*) gene promoter. Later, these researchers compared their findings in a similar study on Hispanics and African-American cohorts. In this report, in non-heroin users (controls), significant differences between the ethnicities were found at the −25 and +12 sites with African Americans showing highest methylation (19.6%), followed by Caucasians (17.4%) and Hispanics (16.1%). When comparing the African American former heroin addicts to controls, significant differences in the +12 CpG site were detected, and it was hypomethylated in former addicts (6.6%) compared to controls (12.2%). In the Hispanic case and control comparison, the −25, −14, and +27 CpG sites were hypermethylated (−25 CpG site 30.5 vs. 23.4%; −14 CpG site 20.4 vs. 15.5%; +27 CpG site: 12.9 vs. 7.7%). No significant associations between methylation of the CpG sites and sex or SNP variants were detected in any of the ethnicities. Increasing DNA methylation with

age was detected at −60 site in Hispanic controls and −18 site in Hispanic cases, and the −10 site in African-American controls. However, adjustments for multiple testing showed that the findings did not affect analyses of ethnic or heroin-related variation in methylation.

When comparing former addicts without history of alcoholism to those with a history of alcoholism, no significant associations were found for DNA methylation level at any of the 16 CpG sites in individuals with history of alcoholism in the African-American former heroin addicts. In contrast, in the Hispanic group, there was a significant association of history of alcoholism with DNA methylation at the −93 site (11.8% in individuals without alcoholism, 8.6% in individuals with alcoholism; $p = 0.020$) and the −60 CpG site (10.5% in individuals without alcoholism, 8.0% in individuals with alcoholism; $p = 0.028$). Their study highlights significant variation in DNA methylation between former heroin addicts and controls specific to Caucasians, African Americans, and Hispanics (Nielsen et al. 2010).

Smoking

In spite of a declining smoking rate in high income countries, smoking-associated illnesses and death remains a severe health issue that is also spreading to low-to-middle income countries. Studies have highlighted correlations between smoking and variation in DNA methylation at distinct loci, which not only distinguish between those who have never smoked and those who currently do, but also reflect the amount smoked. Due to genetics, lifestyle, diet and behavioural differences, the influence of smoking on DNA methylation varies between ethnic groups (Breitling et al. 2011; Elliott et al. 2014; Joubert et al. 2012; Zeilinger et al. 2013). Even prenatal exposure to smoke has been linked to higher levels of methylation (Terry et al. 2008). The *AHRR* gene (aryl hydrocarbon receptor repressor) is among the many genes that have been found to show a significant association with DNA methylation and smoking. The target of *AHRR*, the aryl hydrocarbon receptor (AHR) is a tumour suppressor that facilitates detoxification of carcinogenic agents causing tobacco-related lung cancer. Several methylation sites, including cg05575921 and cg21161138 within the *AHRR* gene, were found to demonstrate significant associations with smoking in Arabs ($p = 7.47 \times 10^{-7}$ and $p = 2.08 \times 10^{-3}$, respectively) (Zaghlool et al. 2015). This was also found in other studies in whole cord-blood samples based on a Norwegian Mother and Child Cohort by Joubert et al. (2012) and Germans by Zeilinger et al. (2013) (cg05575921 −24.40% methylation). Monick et al. (2012) examined DNA methylation of pulmonary alveolar macrophages and lymphoblasts obtained from Caucasian females and found another CpG site associated with smoking, cg14817490 within *AHRR*, which was also detected in Arabs ($p = 3.68 \times 10^{-3}$) by Zaghlool et al. (2015).

Elliot et al. (2014) studied the effect of cigarette smoking on DNA methylation patterns between 96 European and 96 South Asian males of the Southall and Brent Revisited (SABRE) cohort. Methylation levels of cg05575921 within *AHRR* were found to be considerably lower in Europeans than South Asians, which was partly attributed to heavier smoking in the European population (Elliott et al. 2014). Two other loci in *AHRR* showed an interaction between ethnicity and smoking; cg25648203 and cg21161138; the latter in which differential DNA methylation was also identified in Arabs by Zaghlool et al. (2015), Germans by Zeilinger et al. (2013) and the Norwegian Mother and Child Cohort by Joubert et al. (2012).

Similar to other reports, differential DNA methylation was observed at several sites, including *6p21.33, GFI1, F2RL3, ALPPL2,* and *GNG12* (Joubert et al. 2012; Zeilinger et al. 2013; Zaghlool et al. 2015). One specific locus within *TMEM51* that displayed a decrease in methylation was also identified in Germans by Zeilinger et al. (2013). These ethnic differences were attributed to diets, cultural and environmental factors and possible population-specific local mSNPs.

Habitat

In the study by Fagny et al. (2015) that assessed methylation of several African populations ("Association of DNA methylation with genetic and other epigenetic variants in various populations") the habitat of the populations was found to have a significant impact on DNA methylation patterns. Located across 3550 genes and enriched in sites located in gene bodies and distal promoters, 5716 DMRs (independent of genotype differences) were identified upon assessment of methylation variation due to recent changes in habitat between the f-AGR (individuals from a forested region of Cameroon) and w-AGR (those that occupied urban deforested habitats) groups. In the examining of methylation variation of populations with the same current habitat but different historic lifestyles and genetic backgrounds, 4049 DMRs that were located primarily in transcription sites, 5′UTRs and first exon regions of 2128 genes were identified between the f-AGR and w-RHGs. Furthermore, urbanization was found to increase susceptibility to immunity-related disorders. DMRs found between groups that had common historical lifestyle and genetic backgrounds but differed in habitat (f-AGR and w-AGR) were enriched in binding sites of transcription factors located in genes which primarily affected traits such as immune processes (*IRF1, GATA3*), cellular metabolism (*HNF1A, RORA*) and host-pathogen

interactions; and genes associated with autoimmune disorders, such as systemic lupus erythematosus.

In contrast, DMRs found between groups with the same habitat but different historical and genetic backgrounds (f-AGR and w-RHGs) were enriched in transcription factors specifically associated with developmental processes (*NHLH1*). These DMRs were enriched in functions related to development, such as growth factor binding and anatomical structure development. These two independent sets, showing distinct genomic regions that contain diverse transcription factors binding sites, were suggestive of an association to regulatory features interrelated to varying biological functions. While the study by Fagny et al. (2015) was in-depth, informative and provided an array of important biological implications, the measurement of DNA from whole blood is surely a limitation, as stated in their research. DNA methylation in whole blood cells has been found to fluctuate due to an array of external factors such as exposure to carcinogens, chemicals as well as economic status (Fagny et al. 2015; Lam et al. 2012).

Final remarks and future outlook

Epigenetic variations, and in particular, DNA methylation have evidently played key roles not only in differences between individuals, but also between human populations (Fraga et al. 2005; Heyn et al. 2013; Moen et al. 2013) and could contribute to the observed differences in distinct physical appearance, behaviour, and response to environmental agents and drugs. DNA methylation (and other epigenetic changes) provides a biological link between an individual's environmental exposures and their phenotypes and contribute to trans-generational inheritance of complex traits (Trerotola et al. 2015).

There is a strong genetic component to inter-individual variation in DNA methylation signatures. Several methylome-wide studies have provided evidence of individual SNPs that are correlated with specific methylation loci (Bell et al. 2011; Gibbs et al. 2010). Importantly, these studies have shown that SNPs can affect methylation as well as gene expression (McDaniell et al. 2010). The prospect of genetic and epigenetic variation between populations is gaining momentum, especially in the medical community where strenuous efforts have been made to understand the underlying bases of racial health discrepancies. Indeed, it is advantageous to know if individuals within a population are more or less susceptible to diseases and who will most likely benefit from therapeutic interventions (Ku et al. 2010; Lohmueller et al. 2003; Risch et al. 2002; Wilson et al. 2001).

In this regard, a greater understanding of the epidemiology of epigenetic processes will drive development of effective treatments. However, variation in humans cannot be accounted for only by genetic factors (Heyn et al. 2013; Kilpinen and Dermitzakis 2012; Moen et al. 2013).

The present review has thoroughly described several reports of methylation variation between populations within the same geographical location. These studies have proved that habitats, diets and lifestyle factors can substantially alter the methylomes of genetically homogenous populations. Thus, it would be beneficial to examine methylation patterns of populations of similar ethnicities over intercontinental geographical locations, while simultaneously controlling dietary and lifestyle factors. In future, DNA methylation could be targeted to develop markers to track variation between populations. However, to accomplish this, extensive research on transgenerational inheritance of methylation patterns needs to be conducted. Understanding the magnitude of the contribution of epigenetic and environmental factors to variation in gene expression in humans is essential to construe the relationship between epigenotype and phenotype.

While the present review motivates for the concept of differentiation between ethnic groups based on analysis of differential DNA methylation, much debate exists around the concept of categorizing humans by race or ethnicity, irrespective of the biomarker in use. The notion that humans can indeed be classified into various races has been enshrined by some researchers, especially those in the medical community seeking information about disease susceptibility and therapeutic intervention (Fuhrman et al. 2000). However, the concept of human classification is also dismissed by others due to legal and ethical issues. Predominantly, using epigenetic variation for a description or inference of ethnicity of the perpetrator of a crime may lead to stigmatization of certain groups within society, especially concerning skin colour and diseases (Bamshad et al. 2004; Koops and Schellekens 2008). In the forensic world, the use of biological material from a crime scene sample to identify physical traits may initiate controversy because of fears that the technology may be abused. For example, privacy issues arise when sensitive matters such as inclination towards homosexuality and susceptibility to disease are made public (Koops and Schellekens 2008; M'charek et al. 2012). Nevertheless, provided that the DNA analysis is restricted to gaining intelligence related to the case in question and not otherwise, inference of ethnic background by use of DNA methylation markers adds a new dimension in medical research and forensic casework.

Compliance with ethical standards

The authors do not have any conflicts of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

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