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Review

Effects of early-life environment and epigenetics on cardiovascular disease risk in children: highlighting the role of twin studies

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Cardiovascular disease (CVD) is the leading cause of death worldwide and originates in early life. The exact mechanisms of this early-life origin are unclear, but a likely mediator at the molecular level is epigenetic dysregulation of gene expression. Epigenetic factors have thus been posited as the likely drivers of early-life programming of adult-onset diseases. This review summarizes recent advances in epidemiology and epigenetic research of CVD risk in children, with a particular focus on twin studies. Classic twin studies enable partitioning of phenotypic variance within a population into additive genetic, shared, and nonshared environmental variances, and are invaluable in research in this area. Longitudinal cohort twin studies, in particular, may provide important insights into the role of epigenetics in the pathogenesis of CVD. We describe candidate gene and epigenome-wide association studies (EWASs) and transgenerational epigenetic inheritance of CVD, and discuss the potential for evidence-based interventions. Identifying epigenetic changes associated with CVD-risk biomarkers in children will provide new opportunities to unravel the underlying biological mechanism of the origins of CVD and enable identification of those at risk for early-life interventions to alter the risk trajectory and potentially reduce CVD incidence later in life.

Cardiovascular disease (CVD) is the leading cause of death worldwide and carries a huge economic burden (1). Early identification of individuals at increased risk of CVD is fundamental to developing effective prevention. However, known environmental and genetic factors explain only a small proportion of the variability in CVD risk, a major obstacle to prevention (2). This partly reflects the research focus on adulthood rather than early life, a critical but poorly understood period in the pathogenesis of CVD.

The development of atherosclerosis, the underlying pathology of CVD, begins in early life, in some cases before birth (3). An adverse intrauterine environment and impaired fetal growth have been suggested to contribute to the early development of atherosclerosis, with a long latency period between these and other exposures and adult CVD (4). The mechanisms contributing to early CVD risk, however, are unclear. A likely mediator at the molecular level is epigenetic dysregulation of gene expression. Epigenetic factors show interindividual variability at birth that may be stable throughout the life course and have been posited as the likely drivers of early-life programming of adult-onset diseases (5).

Although several recent articles have reviewed current knowledge regarding the role of epigenetics in the pathogenesis of CVD (2,6), evidence regarding the dysregulation of the early-life epigenome (the sum total of genomic epigenetic marks such as DNA methylation) in response to known CVD risk factors has not been summarized. Here, we summarize recent literature on the epidemiology and epigenetics of CVD risk in children, with a particular focus on twin studies. We highlight the use of CVD risk biomarkers in the pediatric populations in risk prediction and targeting of therapy, as well as in the development of interventions that may modulate subsequent risk of CVD.

EARLY-LIFE ORIGINS OF CVD

In the past two decades, evidence has accumulated that early-life risk factors may influence the development of atherosclerosis and subsequent CVD risk in adulthood. Atherosclerosis is a progressive process. The accumulation of lipids in the intima of arteries results in fatty streaks that may develop into atherosclerotic plaques, ultimately causing reduction in blood flow to critical organs, especially if the plaques rupture. Autopsy studies have shown a higher frequency of fatty-streak lesions detected in the arteries of human fetuses from mothers who smoked heavily in pregnancy or those with hypercholesterolemia as compared with those unexposed (7). Furthermore, fatty streaks detected in aortas in children as young as 3 y progress to atherosclerotic plaques by young adulthood (8).

The hypothesis, suggested by Barker and colleagues, that initially focused on adverse maternal environment, low birth

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weight, and CVD risk (9) has evolved into the "Developmental Origins of Health and Disease" hypothesis. This emphasizes the critical early-life period when nutrition and other environmental stimuli may influence developmental pathways and induce long-lasting changes in metabolism and cardiovascular health (4), although the causal relationships and underlying mechanisms remain controversial.

Pediatric studies on environmental factors that may influence risk of CVD have investigated a range of CVD biomarkers (10). A range of CVD risk factors and vascular health biomarkers that have been measured in childhood are summarized in the **Table 1**. Vascular endothelial dysfunction begins in early life and is a central pathological status in the early development of atherosclerosis (11). The role of biomarkers of endothelial function in CVD development has thus been investigated. In line with this, early-life CVD risk factors, in particular childhood obesity, are strongly associated with endothelial dysfunction and the development of atherosclerosis and CVD (12). More important, there is emerging evidence that childhood CVD risk factors, such as hypertriglyceridemia, are independently predictive of adult CVD (13), although studies have been relatively underpowered and larger sample sizes are warranted (14). There is also strong evidence that CVD risk factors in childhood are associated with CVD precursor conditions or intermediate vascular end points in adulthood (e.g., diabetes, hypertension, and larger carotid intima-media thickness) (15). Clustering of CVD risk factors, which imparts an even greater risk than the sum of the individual risk factors in adults (16), also occurs in childhood (17,18). This clustering of CVD risk factors not only tracks from childhood into adult life (19) but is also associated with the antecedents of increased blood pressure and skinfold thickness in early infancy (18).

By the preschool years, low-birth-weight infants have early signs of impaired vascular health, including thicker carotid intima-media thickness (3) and increased arterial stiffness (20). These infants also have higher levels of CVD risk factors such as adiposity, insulin resistance, and dyslipidemia as compared with those with normal birth weight (21). The relationship between compromised intrauterine nutrition and low birth weight, however, is not straightforward, and other adiposity measures such as ponderal index (weight divided by length cubed) and abdominal circumference at birth may be better early-life predictors of adult CVD risk than birth weight alone (22). Growth velocity in early postnatal life, particularly rapid "catch-up" growth during the first few months in low-birth-weight infants and weight gain in childhood, strongly affects endothelial function and subsequently the development of atherosclerosis and CVD (22). It has been proposed that childhood weight gain diverts energy disproportionately to adipose tissue, particularly in the abdomen, thereby increasing metabolic load (23). In addition, animal studies have shown that nutritional "mismatch" may occur when intrauterine undernutrition is followed by postnatal overnutrition, especially with energy-dense foods (4).

Table 1. CVD-risk biomarkers studied in children

Biomarker	Parameters measured
Measures of vascular health	
Carotid (and aortic) intima-media thickness	Subclinical atherosclerosis
Arterial distensibility, compliance, elastic modulus, and pulse wave velocity	Arterial stiffness
Flow-mediated vasodilatation	Endothelial function
High-sensitivity C-reactive protein	Systemic inflammation
Serum ICAM-1 and VCAM-1	Endothelial function
Retinal arteriolar and venular caliber	Diameter of microvasculature
Traditional CVD risk factors	
Adiposity	BMI, waist circumference, skinfold thickness, body fatness, ponderal index, and so on
Serial weight gain	Weight for age at birth, and so on
Systolic and diastolic blood pressure	Blood pressure
Serum HDL cholesterol, total cholesterol	Dyslipidemia
Serum apolipoprotein B	Dyslipidemia
HOMA-IR	Insulin resistance

CVD, cardiovascular disease; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

VASCULAR HEALTH BIOMARKERS, CVD RISK FACTORS, AND CVD RISK IN NONTWIN CHILDREN

Measures of Vascular Health

Various measures of vascular health, including carotid intima-media thickness, pulse wave velocity, flow-mediated vasodilatation, high-sensitivity C-reactive protein, and cell adhesion molecules, have been suggested as valuable biomarkers of vascular health in pediatric epidemiological studies (10,24). Current data have demonstrated that in pediatric populations, adverse changes in these biomarkers are associated with a range of CVD risk factors, including elevated blood pressure, type 1 and 2 diabetes, obesity, and hypercholesterolemia. Therefore, these biomarkers may be useful in the detection of early structural and functional vascular damage in children with increased CVD risk.

The retinal vasculature, another biomarker of vascular health in children, can be directly and noninvasively quantified (25). Data from several adult prospective population-based studies suggest that retinal arteriolar and venular caliber (diameter) changes are predictive of the incidence of CVD, independent of traditional risk factors (26). Limited epidemiological pediatric studies have shown that retinal vessel caliber is associated with CVD risk factors such as elevated blood pressure (27) and adiposity (28). However, the cross-sectional nature of these studies does not allow for causal inferences. A recent study also demonstrates that poor growth *in utero* may have an adverse influence on retinal vasculature in children (29).

Maternal Environment During Pregnancy and Traditional CVD Risk Factors

Gestational diabetes, maternal smoking, and maternal obesity are putative early-life predictors of adult CVD. Longitudinal cohort studies have shown that maternal obesity increases the

risk of metabolic syndrome in offspring during childhood by approximately twofold (30) and that maternal smoking during pregnancy is associated with CVD risk factors (e.g., metabolic syndrome, elevated blood pressure) in offspring (31). In a systematic review and meta-analysis, it was found that prolonged breastfeeding (≥4 mo) may be protective against childhood CVD risk factors such as elevated blood pressure (32). However, these effects were mainly observed in small studies, with little evidence from larger studies (≥1,000 participants).

CVD BIOMARKERS AND CVD RISK IN TWIN CHILDREN

Studies of monozygotic (MZ, genetically identical) and dizygotic (DZ, sharing half of their genetic variation) twins provide a natural study design to assess the relative contribution of nature (heredity) and nurture (environment) on the variation of traits and complex diseases, including CVD (33). Twins are inevitably matched for age, closely matched for (shared) prenatal (e.g., length of gestation, maternal nutrition) and postnatal environment (e.g., rearing). Classic twin studies enable partitioning of phenotypic variance within a population into additive genetic (also known as heritability) and shared environmental and nonshared environmental effects, which include factors that are specific to each fetus, umbilical cord, and placenta. Twin studies using phenotype-discordant MZ twins, in particular, are one of the most powerful study designs in epigenetic epidemiology (34).

Do Twins Always Share an Equal Environment?

Despite having the same mother, twin pairs may experience different intrauterine environments (35). All DZ twins and one-third of MZ twins have separate amnions and chorions, and their placentas may be either separate or fused. The remaining MZ twins are monochorionic (sharing a common chorion and placenta). Roughly 50% of the twins with separate chorions have fused placentas, resulting in vascular anastomoses in a large proportion of MZ and 5–8% of DZ twins (36). Such differences in the nutritional "supply line" between mother and each fetus may ultimately result in birth weight discordance.

Twin studies have revealed differing influences of genetic, shared, and nonshared environmental factors on a range of CVD biomarkers. Suggestions that findings from twin studies may not be generalizable have largely been refuted, although differences may exist in very early childhood (37). In addition, the classic twins design can be improved by including nontwin siblings and parents (37).

A small number of twin studies provide consistent evidence that the nonshared intrauterine environment plays a key role in the development of type 2 diabetes and metabolic syndrome, with implications for CVD risk. A study of 104 pairs of 8-y-old twins strongly indicated that a critical factor underlying the association between low birth weight and high blood pressure must involve nonshared environment (38). A recent study also demonstrated an independent within-pair association between smaller birth size and narrower retinal arterioles in both MZ and DZ twin pairs aged 5–14 y (39), supporting the notion that twin-specific supply line factors affect fetal growth and vascular health. In 31 pairs (22 MZ and 9 DZ) aged 4–12 y, it was shown that lower birth weight is associated with higher systolic blood pressure, endothelial dysfunction, and thicker carotid intima-media thickness (40). A study of 114 (53 DZ and 61 MZ) adolescent twin pairs by Ijzerman *et al* found that lower birth weight was associated with insulin resistance, lower high-density lipoprotein levels, and shorter height in both DZ and MZ twin pairs, again implicating a large nonshared environmental component in their variation (41). This study also found that low birth weight was associated with blood pressure, total and low-density lipoprotein cholesterol, and fibrinogen within DZ but not MZ twin pairs, suggesting an additional genetic influence on these associations (41). A longitudinal adolescent twin study (125 MZ and 166 DZ pairs) involving 965 families, however, found little evidence of shared environment effects on the variation in lipids, except high-density lipoprotein cholesterol, and only minimal effects of nonshared environment (42). A twin study involving 174 twin pairs showed that maternal alcohol exposure during pregnancy was associated with increased carotid-femoral pulse wave velocity but not with systolic or diastolic blood pressure or child adiposity measures (e.g., BMI) at 9 y (43).

EPIGENETICS AND ITS ROLE IN DEVELOPMENTAL PROGRAMMING OF CVD

Epigenetics describes mechanisms that stably control gene activity through multiple rounds of cell division (44). The best-understood epigenetic mechanism is DNA methylation of the cytosine base of the CpG dinucleotide (45) (**Figure 1**). Other epigenetic mechanisms include covalent modification of DNA-packaging histone proteins and noncoding RNA. DNA methylation has context-dependent effects on transcriptional regulation and efficiency; although it is generally associated with a reduction of expression when present in gene promoters, there is emerging evidence that it can have a location-dependent association with expression, elongation, splicing, and silencing of repetitive DNA (45).

Epigenetic change has been strongly implicated as a key mechanism in the early-life origins of CVD (2). In the pre- and early postnatal period, the epigenome is at its most dynamic and may alter in response to environmental stimuli as reviewed by Szyf (46). A number of intrauterine environmental factors (e.g., maternal diet) have been associated with epigenetic modification, providing a plausible mechanistic link between early-life exposures and changes to the epigenome and adult CVD (47). More important, inflammation, the central pathological process involved in atherosclerosis, has also been associated with global hypermethylation (48). Animal studies, for example, a study conducted by Weaver *et al.* (49), suggest that these epigenetic modifications may be reversible in early life by appropriate pharmacological agents or even targeted dietary interventions.

ANIMAL STUDIES OF ENVIRONMENT-INDUCED EPIGENETIC PROGRAMMING OF CVD RISK

Animal studies of the early-life origins of CVD have focused on the effect of maternal dietary restriction during gestation on

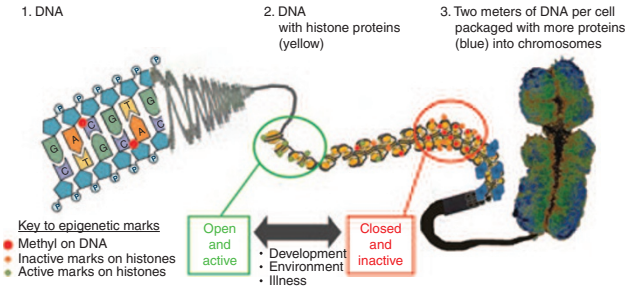

Figure 1. Epigenetic mechanisms: from DNA to chromosomes. In each nucleated cell, DNA is packaged with histone proteins and then further packed with more proteins to form chromosomes. The most commonly studied epigenetic marks, DNA methylation (at the CpG dinucleotide) and histone modifications, which act in concert to influence local DNA packaging, are shown. Combinations of epigenetic marks determine the tightness of this packaging, which in turn influences gene activity via access to the cellular machinery involved in gene transcription and higher-order genomic interaction between regulatory regions such as promoters and enhancers. Regulatory regions can switch between open/active and closed/inactive as part of normal development, in response to environment, or in association with illness.

expression and methylation of key metabolic and cardiovascular regulator genes in tissues such as liver, heart, adrenal gland, and kidney in rats and pigs (50). Offspring exposed to altered maternal nutrition *in utero* showed modulation of gene expression in adult life associated with subsequent hypertension and dyslipidemia (51). Specific genes showing disrupted DNA methylation include *Ppara* (peroxisome proliferator–activated receptor-α), a major regulator of lipid metabolism (50); *Nr3c1* (the glucocorticoid receptor), a key regulator of metabolism (50); and *Pepck* (phosphoenolpyruvate carboxykinase) and *Hmgcr* (HMG-CoA reductase), which catalyse rate-controlling steps in gluconeogenesis (52) and cholesterol production (50), respectively. These data suggest that dietary change in pregnant mothers alters DNA methylation and gene expression of metabolic regulatory genes in key tissues, which may permanently alter the structure and function of these tissues, causing metabolic and cardiovascular dysfunction and predisposing to later CVD. Similar protein restriction models have also found changes of expression of renin and angiotensin (53), the angiotensin receptor (54), and *Dnmt1*, a key DNA methylation enzyme (55). Of note, changes in DNA methylation of key upstream regulators have been linked to both altered expression of target genes (*Ppara* and *Nr3c1*) and concomitant changes in downstream target genes (*Aox* and *Pepck*) with subsequent phenotypic changes (hepatic β-oxidation and gluconeogenesis) (52,55).

HUMAN STUDIES

A small number of human studies have linked adverse prenatal environment, epigenetic change, and CVD risk. For example, middle-aged offspring of mothers exposed to the Dutch Famine during early pregnancy showed differences in DNA methylation in metabolic and CVD-related candidate genes such as those encoding leptin (*LEP*) and insulin-like growth factor 2 (*IGF2*) as compared with their unexposed siblings (47). Although these studies did not link DNA methylation to gene expression, the regions studied are known to correlate with gene expression (47). Offspring exposed to intrauterine malnutrition had a higher adult BMI (56), increased lipids (57), and increased CVD risk (58). Lower maternal carbohydrate intake in early pregnancy was associated with changes in DNA methylation of retinoid receptor-α (*RXRA*) and endothelial nitric oxide synthase (*NOS3*) at birth, which in turn correlated with later childhood adiposity (59). The strength of this study is the relationship between *RXRA* methylation at birth and childhood adiposity and the replication in another cohort. However, no attempts were made to correlate methylation with gene expression. Other gestational CVD risk factors shown to influence DNA methylation in humans include maternal smoking (60), folate intake (61), and levels of homocysteine (62), which is involved in the development of atherosclerosis by inducing changes in DNA methylation in multiple genes in vascular smooth muscle cells and is the biomarker most directly implicated in epigenetic mechanisms relating to CVD risk (2). Epigenetic marks at these genes are sensitive to the prenatal environment (63). Although these studies do not look at whether changes in DNA methylation correlate with changes in gene expression, these data suggest that epigenetic modification is an important underlying mechanism contributing to the link between early-life environment and later CVD risk.

ASSOCIATION OF DNA METHYLATION WITH CVD-RISK FACTORS IN HUMANS

The mechanistic role of epigenetics in CVD is incompletely understood but is a focus of intense research interest. Genomic DNA isolated from human atherosclerotic lesions is hypomethylated as compared with that of normal tissue (64). More studies are needed to clarify these relationships, although gene-based studies are more likely to lead to the development of appropriate therapeutic agents. In adults, altered DNA methylation at genes including those encoding insulin (*INS*) and GNAS antisense RNA 1 (*GNASAS*) is associated with risk of myocardial infarction, although there was no investigation of the effect of altered DNA methylation on gene expression (63). It was also shown recently that childhood obesity is associated with hypermethylation of the proopiomelanocortin (*POMC*) gene involved in energy homeostasis and in which genetic polymorphisms are associated with early-onset obesity and adrenal insufficiency (65). Further clues to the mechanisms of type 2 diabetes have come from recent studies showing that transient hypoglycemia can result in a permanent epigenetic change, with altered histone modification associated with changes in gene expression (reviewed by Tonna *et al.*, ref. 66).

These data linking locus-specific epigenetic modification with CVD risk factors are derived largely from epigenetic candidate gene approaches. However, the majority of phenotypic variation due to DNA methylation will not be detected by this approach, and epigenome-wide association studies (EWASs) have recently been advocated (67). No EWAS of CVD *per se* has been performed. However, a limited number of EWASs have been performed on CVD intermediate phenotypes or risk factors. An EWAS using massively parallel sequencing coupled

with immunoprecipitation to analyze DNA methylation and histone modification in human aortic endothelial cells exposed to transient hypoglycemia found an association of epigenetic alteration and expression changes in genes involved in metabolic and cardiovascular health (68). A study comparing DNA methylation in pancreatic tissue from five adults with type 2 diabetes who died and 11 matched controls found differential methylation in genes related to β-cell survival and function (69). The authors found corresponding changes to expression in a subgroup of differentially methylated genes. In whole blood from adults with diabetic nephropathy (as compared with normal controls), several differentially methylated CpG sites in biologically plausible genes were observed (70). Nineteen differentially methylated CpG sites were observed that associated with diabetic nephropathy. Although no single group of genes stood out, one of the associated genes, *UNC13B*, whose product binds diacyl glycerol, is associated with diabetic nephropathy. Another recent study using expression and methylation arrays (~1,500 CpGs with limited genomic coverage) found that DNA methylation in cord blood is associated with altered gene expression, body size, and body composition (71). In addition, Feinberg *et al* identified four regions of the genome at which DNA methylation covaried with BMI at two time points during adulthood (5). These regions included genes such as that encoding matrix metalloproteinase 9 (*MMP9*), previously associated with metabolism and obesity. The effect of methylation on gene expression, however, was not examined.

Twin Studies

As epigenetic modifications such as DNA methylation can be considered quantitative traits (72), twin studies offer a unique opportunity to investigate the heritability of epigenetic modifications. Studies of adult twins have found that at least for a proportion of the genome, DNA methylation can have a high heritability (73). Our findings from the Peri/postnatal Epigenetic Twins Study highlight the influence of nonshared intrauterine environment, genetic variations, and shared environment on epigenomic profile (74–76). Analysis of DNA methylation in MZ and DZ pairs revealed that the largest component of variation was attributed to the combined effects of nonshared intrauterine environment and stochastic factors, highlighting the importance of the intrauterine environment on shaping the neonatal epigenome (76). We also used within-pair birth weight discordance to analyze genomic regions at which gene expression (75) and DNA methylation (76) correlated highly with birth weight and such genes were enriched in those associated with CVD risk. These findings show that twins are an ideal model to identify components of variation in DNA methylation at birth and beyond, and enable the differentiation between shared and nonshared environmental influences.

To our knowledge, only one EWAS of a CVD-related phenotype has been performed in twins. Using DNA methylation profiles of purified CD14+ monocytes from 15 type 1 diabetes–discordant pairs of MZ twin children (77), this study revealed the presence of 132 genomic locations that differed in DNA methylation in all twins with type 1 diabetes. Genes associated with these CpGs were enriched in those involved in immune function including HLA-related genes (*HLA-DQB1*) and regulatory factor X-associated protein (*RFXAP*), both previously associated with type 1 diabetes in genetic studies, and the proinflammatory cytokine tumor necrosis factor. However, this study is limited by a small sample size, the lack of gene expression data, and the use of whole blood, a tissue for which differential methylation of its cellular constituents could influence overall DNA methylation.

TRANSGENERATIONAL EPIGENETIC INHERITANCE OF CVD

Epigenetic marks influenced by intrauterine environment are classified as transgenerational epigenetic effects because a maternal environmental factor can have epigenetic effects or even epigenetically independent toxic effects on the developing fetus (offspring or the F1 generation) and, if the fetus is female, on developing germ cells that go on to contribute to the grandchildren (the F2 generation) (78,79). True transgenerational epigenetic inheritance can occur only when environmentally induced epigenetic changes survive the genome-wide epigenetic remodeling that accompanies gametogenesis and early postzygotic development, e.g., epigenetic changes observed in the great-grandchildren (the F3 generation) in the example above, as has been demonstrated for rats exposed to endocrine disruptors (80). Transgenerational epigenetic inheritance has been demonstrated in a variety of organisms, such as plants, mice, and fission yeast. Human studies have also pointed to epigenetic inheritance (81), although conclusive evidence is lacking; in such cases, an inherited genetic influence on epigenetics has to be ruled out (82). Circumstantial evidence from human epidemiological studies has shown that smoking or dietary exposures in previous generations are associated with BMI in 9-y-old sons (83) and with adult mortality from CVD and type 2 diabetes (84). A critical exposure time appears to be the prepubertal slow growth period, which coincides with testicular descent and epigenetic reprogramming in merging pools of spermatocytes (85). Direct evidence of transgenerational epigenetic inheritance of CVD-related phenotypes comes from animal studies. Male mice fed a low-protein diet fathered offspring with lower hepatic expression levels of genes involved in lipid and cholesterol biosynthesis, including *Ppara*, which also exhibited DNA methylation changes (86). Male rats fed a high-fat diet fathered obese sons, who had altered pancreatic gene activity, which at one gene, the interleukin receptor *Il13ra2*, was accompanied by a large alteration in DNA methylation (87). Furthermore, male pigs fed a diet high in "methylating micronutrients" including folate, vitamin B12, and methionine had leaner grandchildren that also had changes in DNA methylation and gene expression in muscle, liver, and kidney, including the iodotyrosine deiodinase (*Iyd*) gene involved in thyroid function (88).

ROLE OF EPIDEMIOLOGY IN EPIGENETIC RESEARCH OF CVD

We have not attempted to cover comprehensively the contribution of epidemiology in defining the role of epigenetics in CVD, which has been the focus of several recent reviews

(e.g., refs. 89,90). However, a number of important concepts have emerged that need to be taken into account when conducting such studies. Most important, epigenetic changes within an individual can vary over life span, and epigenomic profiles should be considered phenotypes in epigenetic epidemiological designs rather than genotypes. Epigenetic variation can therefore be causal for, or be a consequence of, a trait/disease. It is therefore difficult to establish causality using a large number of associations generated from epigenetic association studies. Longitudinal cohort study designs that have obtained epidemiological data and biological samples from initially disease-free individuals (ideally before or from birth) over the life course could clarify such issues of reverse causality (67). Associations of epigenetics with disease phenotypes may be influenced by confounding (e.g., by socioeconomic status), as can happen with conventional observational epidemiological studies (89). New analytic strategies have been developed to interrogate the causal relationships among environmental exposures, DNA methylation, and outcome. Some of these, if certain conditions are met, utilize genetic variants to act as an instrumental variable to help assess or account for potential confounding (91).

RESEARCH INTO EVIDENCE-BASED INTERVENTIONS

Animal studies have shown that the epigenome can still be in flux during early postnatal life and that adverse epigenetic modification can be reversed by postnatal diet such as methionine or epigenetic inhibitors such as trichostatin A (49). Furthermore, neonatal leptin treatment can reverse the detrimental effects on offspring health resulting from a maternal high-fat diet (92). A rodent study also showed that offspring fed a postnatal diet enriched with ω-3 fatty acids attenuated prenatally programmed hypertension and hyperleptinemia (93). Such results bode well for future interventions in humans but only after accumulation of evidence from independent laboratories and if humans respond in similar ways as animals. One recent example of cross-cohort validation came from two independent human EWASs that identified changes in the same genes in offspring following exposure to maternal smoking during pregnancy (94). In summary, identification of epigenetic biomarkers in childhood that may predict adult CVD and related diseases could lead to better ways to monitor progress of interventions, whether these are developed through epigenetic knowledge or not.

CONCLUSION

Identification of epigenetic changes associated with biomarkers of CVD risk in children will offer new opportunities to unravel the underlying biological mechanism of early-life origins of CVD. Longitudinal cohort twin studies, in particular, may contribute to the understanding of the role of epigenetics in the pathogenesis of CVD. As current risk-stratification strategies for CVD are suboptimal, novel early-life CVD-risk biomarkers might improve these strategies, enabling early identification of those at risk and facilitating early-life interventions to alter the risk trajectory and potentially reduce the incidence of CVD later in life.

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