Fetal programming of chronic kidney disease: the role of maternal smoking, mitochondrial dysfunction, and epigenetic modification

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Stangenberg S, Chen H, Wong MG, Pollock CA, Saad S. Fetal programming of chronic kidney disease: the role of maternal smoking, mitochondrial dysfunction, and epigenetic modification. Am J Physiol Renal Physiol 308: F1189–F1196, 2015. First published February 5, 2015; doi:10.1152/ajprenal.00638.2014.—The role of an adverse in utero environment in the programming of chronic kidney disease in the adult offspring is increasingly recognized. The cellular and molecular mechanisms linking the in utero environment and future disease susceptibility remain unknown. Maternal smoking is a common modifiable adverse in utero exposure, potentially associated with both mitochondrial dysfunction and epigenetic modification in the offspring. While studies are emerging that point toward a key role of mitochondrial dysfunction in acute and chronic kidney disease, it may have its origin in early development, becoming clinically apparent when secondary insults occur. Aberrant epigenetic programming may add an additional layer of complexity to orchestrate fibrogenesis in the kidney and susceptibility to chronic kidney disease in later life. In this review, we explore the evidence for mitochondrial dysfunction and epigenetic modification through aberrant DNA methylation as key mechanistic aspects of fetal programming of chronic kidney disease and discuss their potential use in diagnostics and targets for therapy.

maternal programming; chronic kidney disease; mitochondrial dysfunction

CHRONIC KIDNEY DISEASE (CKD) is a major health burden. One in six adults over the age of 25 yr shows some degree of CKD with increasing incidence in more advanced age (68). The prevalence of end-stage kidney disease continues to increase (20) and is not always explained by traditional risk factors. It often remains unclear why the rate of CKD progression shows substantial variation from patient to patient even among individuals with similar comorbidities. The theory of fetal programming which links chronic adult diseases to adverse conditions in early life is an intriguing concept, which is gaining increasing attention. Low birth weight (LBW) has been used as a clinical surrogate for a poor intrauterine environment. Numerous epidemiological studies have demonstrated an association of LBW to increased risk of adult-onset diseases such as cardiovascular disease, hypertension, type 2 diabetes mellitus, obesity, and renal disease (4, 5, 24, 32, 45, 46). White et al. (79) indicated in a systematic review that individuals born with LBW have a 70% relative risk increase of developing CKD in later life.

Impaired nephrogenesis resulting in reduced nephron number is the postulated mechanism linking LBW with subsequent hypertension and risk of CKD (12). Autopsy studies have supported the notion that hypertension is associated with low nephron endowment (35, 40). Nephron deficiency in animal models is frequently associated with renal disease in later life evidenced by lower glomerular filtration rate, azotemia, proteinuria, and glomerulosclerosis (29, 30, 71). Studies on children with oligomeganephronia (22, 50) and in the Aboriginal population of Australia who have high rates of CKD confirm the link between reduced nephron number and progressive renal injury (34). It is hypothesized that reduced nephron number causes glomerular hyperfiltration and compensatory hypertrophy (12, 13), possibly at the expense of increased intraglomerular pressures and subsequent glomerulosclerosis. The underlying molecular mechanism of a reduced nephron endowment remains unknown. Moreover, as some experimental models of congenitally reduced nephron endowment have failed to demonstrate hypertension and glomerular sclerosis (10, 33), it suggests that low nephron number is unlikely the sole risk factor for CKD susceptibility in later life and additional factors are involved in programming of hypertension and renal disease.

Maternal Smoking and Fetal Programming

Maternal smoking during pregnancy remains the most common modifiable adverse fetal exposure, leading to LBW and other adverse perinatal outcomes (3, 36). Despite an encouraging decrease in population-wide smoking rates over the last decade, smoking in pregnancy is still prevalent. According to the 2010 Pregnancy Risk Assessment and Monitoring System (PRAMS) data from 27 states in the United States, ~10.7% of...
women reported smoking during the last 3 mo of pregnancy. Data from 10 PRAMS states were available between 2000 and 2010 and showed only a small reduction of smoking during pregnancy from 13.3% in 2000 to 12.3% in 2010 (77). Cigarette smoke contains more than 4,000 toxins, many of which are able to cross the placenta. Nicotine levels are higher in the amniotic fluid, fetal serum, and placenta than in the corresponding maternal serum (48). These toxins can exert a direct toxic effect on fetal cells or indirectly affect the fetus through morphological and functional changes in the placenta, which include but are not limited to thickening of the trophoblastic membrane, changes in placent al enzymatic functions, and placental vasoconstriction (38). Maternal smoking can thus influence fetal development, and it is not surprising that adverse consequences can be seen in various organ systems in the offspring even at a later age. Studies have shown that smoking in pregnancy is associated with increased body mass index in adult offspring even after adjustment for postnatal confounders (49, 59). An association of maternal smoking during pregnancy and development of hypertension in the offspring has been demonstrated in several studies, and this effect was independent of birth weight, suggesting that fetal nicotine exposure results in long-term cardiovascular consequences (8, 53). A recent review by Duskova et al. (23) nicely discusses the evidence for smoking-induced endocrine changes affecting pituitary hormones, cortisol, estrogens, and androgens in the offspring, which may influence reproductive ability in later life. Smoke exposure during gestation and lactation in mice resulted in altered renal protein expression in the offspring. Markers of inflammation and components of cell-to-cell signaling, lipid metabolism, cell cycle, nucleic acid, and carbohydrate metabolism were involved (37). The detrimental effect of maternal smoking on kidney development was investigated in a retrospective cohort study among 1,072 children. Taal et al. (75) demonstrated that smoking >10 cigarettes/day was associated with smaller kidney volume in fetal and postnatal life. It is difficult to assess the level of smoke exposure (dose) required to induce these effects. In the study by Taal et al., smoking <5 cigarettes/day was associated with larger kidney volume whereas smoking more >10 cigarettes/day resulted in small kidney volume. The number of cigarettes smoked may be difficult to ascertain due to underreporting and nondisclosure, which was as high as 22.9% among pregnant women in one study (21). On the other hand, the measurements of serum or urinary cotinine levels are unlikely a true reflection of exposure throughout pregnancy, especially if collected before birth when most smoking mothers often reduce their nicotine intake (23).

In addition, the dose and design of smoke exposure in experimental models vary greatly, preventing us from making a final conclusion regarding the dose relationship. The results from Taal et al. (75), however, were recently corroborated by MRI imaging in pregnant women who smoked a mean of 9 cigarettes a day showing reduced kidney volume (2). As there has been a documented association of neonatal kidney volume and nephron number (83), it is necessary to investigate whether maternal smoking leads to a susceptibility to CKD in adult offspring. However, studies investigating this link are scarce. We have shown in an animal model that smoke exposure during gestation leads to delayed nephron development and reduced nephron numbers as well as albuminuria at an adult age (1). A recent study by Chen et al. (18) in rats even demonstrated renal fibrotic changes in offspring whose mothers were exposed to subcutaneous nicotine injection during gestation. The underlying cellular mechanisms remain to be elucidated.

Mitochondrial Dysfunction as a Result of Maternal Smoking

There is in vivo and in vitro evidence that maternal nicotine exposure during pregnancy results in increased oxidative stress in fetal and neonatal tissues (25, 52). Mitochondria are the major source, but also a primary target, of reactive oxygen species (ROS), which are generated as byproducts of ATP synthesis through the oxidative phosphorylation system (OXPHOS) in the electron transport chain (ETC). Mitochondria serve a crucial role in development by providing energy for rapid fetal growth and play key roles in cell signaling. Toxin exposure during this phase in life resulting in mitochondrial dysfunction is likely to have long-lasting effects and may lead to failure of organ function over time. It has been known for nearly two decades that maternal smoking induces structural changes in mitochondria in the fetus. These mitochondria appear more elongated or swollen and have less distinct cristae (9). More recent studies have found that these structural abnormalities translate into mitochondrial dysfunction. Broin et al. (16) demonstrated in rodent models that fetal nicotine exposure alters mitochondrial structure and activity of the OXPHOS enzyme complex IV in pancreatic tissues. These mitochondrial changes were observed as early as 3 wk after birth (weaning age) and progressively increased even though nicotine exposure was discontinued at weaning. Importantly, these changes preceded an impaired glucose tolerance in adult life, highlighting the role of fetal programming in the development of chronic adulthood diseases (16). Similarly, in an atherosclerosis mouse model using apolipoprotein E null mice, Fettiman et al. (25a) demonstrated that in utero smoke exposure significantly altered mitochondrial DNA copy number and caused mitochondrial DNA deletions and this was associated with the development of atherosclerosis in adulthood. The adverse effect of maternal smoking on mitochondrial function in the offspring has also been investigated in human placenta, which is predominantly comprised of fetal tissue. Bouhous-Noet et al. (11) observed a significant reduction in enzymatic activity of OXPHOS complex III with a negative correlation between enzyme activity and number of cigarettes smoked per day. Despite the kidney being a highly metabolic organ and rich in mitochondria, an effect of maternal smoking on renal mitochondria in the offspring has not been investigated. It certainly warrants further studies, especially now that the role of mitochondrial dysfunction in renal pathophysiology is increasingly recognized.

Mitochondrial Dysfunction in CKD

Evidence for mitochondrial dysfunction in CKD and hemodialysis patients has recently emerged from high-throughput genome-based microarray technology. Granata et al. (31) screened blood samples in this cohort revealing differential expression of 44 of the tested 12,357 genes, with 25% of these genes encoding for mitochondrial OXPHOS. Enzymatic activity for OXPHOS complex IV was reduced in the CKD and hemodialysis patients compared with control (31). Proteinuria is one of the risk factors for progression of CKD and is
commonly caused by podocyte injury. Podocytes are highly energetic cells that form part of the glomerular filtration barrier and contain an abundance of mitochondria. Mitochondrial dysfunction has been implicated as an early event in aldosterone-induced podocyte injury (72, 81, 84). In addition, mitochondrial dysfunction is involved in epithelial-to-mesenchymal transition (EMT), which is a major mechanism in renal tubulointerstitial fibrosis, the histological hallmark of advanced CKD (80, 82). Exposure of human renal proximal tubular epithelial cells to ethidium bromide caused reversible depletion of mitochondrial DNA with reduction of OXPHOS activity and induced EMT in these cells. Following ethidium bromide removal, mitochondrial DNA recovered and EMT was reversed (80). Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is the major regulator of mitochondrial biogenesis. It is also required for the induction of many ROS-detoxifying proteins including glutathione peroxidase, catalase, uncoupling protein 2, and superoxide dismutase 2 (SOD2), thereby playing a key role in protective pathways of oxidative stress and mitochondrial dysfunction. Induction of PGC-1α results in a robust increase in mitochondrial number, cellular respiration, and intracellular ATP concentration. Experimental overexpression of PGC-1α prevented aldosterone-induced mitochondrial dysfunction and inhibited EMT in human kidney cell lines while also ameliorating podocyte injury (80, 81). An increasing body of evidence indicates that impaired mitochondrial homeostasis may also be a determinant in the development and progression of diabetic nephropathy (26, 65, 67). An underlying mitochondrial dysfunction may explain why 30% of patients with diabetes develop diabetic nephropathy, whereas the remainders don’t. Recently, Sharma et al. (65) performed urine metabolomics from patients with diabetes and CKD (DM+CKD) vs. diabetic patients without CKD (DM−CKD) and healthy controls, revealing differential expression of 13 metabolites in the DM+CKD group. The majority of these metabolites are linked to mitochondrial metabolic. Validation of these pathways revealed that protein levels of OXPHOS complex IV were decreased in diabetic nephropathy biopsies along with a reduction of mitochondrial DNA content in urine exosomes. The downregulation of PGC-1α mRNA in diabetic nephropathy biopsy samples compared with nondiabetic individuals suggests that mitochondrial biogenesis may be impaired as well (65).

Mitochondrial Dysfunction in Acute Kidney Injury

There is also emerging evidence that mitochondrial dysfunction is a key contributor in acute kidney injury (AKI). Recent studies in experimental animal models and on human biopsies have demonstrated that cell death in septic AKI is disproportionately low despite often severely impaired renal function (76, 78). Subtle vacuolization in proximal tubular cells are often the only documented structural lesions and may actually represent swollen mitochondria (76, 78). Equivalently, in ischemia-reperfusion injury, mitochondrial swelling within tubular cells have been reported as the only detectable structural change (55, 57). Hence, the most widespread change in AKI seems to be on a subcellular and molecular level involving mitochondrial function rather than a histopathological change. Several studies have shown a reduction of the mitochondrial OXPHOS enzymes and their activity in experimental models of AKI (27, 56, 78). Tran et al. (78) attributed endotoxin-induced AKI to mitochondrial dysfunction in the kidney, accompanied by reduced expression of genes encoding OXPHOS enzymes and diminished expression of the key mitochondrial biogenesis regulator PGC-1α. PGC-1α expression was proportionally suppressed with the degree of renal impairment and was highly correlated to downstream targets involved in electron transport and other mitochondrial processes. To address whether PGC-1α changes were contributing or were downstream of the change in glomerular filtration rate (GFR), Tran et al. examined renal function in a septic AKI model of global and tubule-specific PGC-1α knockout mice. Both strains had baseline renal function comparable to controls but sustained loss of GFR following sepsis in the knockout strain, implicating PGC1-α-mediated mitochondrial biogenesis as a key factor for recovery in AKI. Additional studies have shown depletion of OXPHOS proteins in rodent models of AKI and support the concept of PGC-1α-mediated recovery of mitochondrial and cellular functions (27, 61).

As discussed above, an unfavorable in utero milieu such as induced by maternal smoking can cause fetal programming of mitochondrial dysfunction. In the kidney, this may be subclinical without obvious symptoms, but it is tempting to speculate that in the setting of other insults such as sepsis, toxins, or ischemia-reperfusion injury, it may predispose to more severe forms of AKI, or irreversible damage that leads to CKD. Further studies to investigate this hypothesis are warranted.

Epigenetic Modification in Kidney Diseases

Epigenetic modifications provide another potential mechanism for how environmental influences in early life cause a susceptibility to certain diseases in adulthood. Epigenetic mechanisms include DNA methylation, histone modification, and noncoding RNAs, which confer changes in gene expression without altering DNA sequence. Among epigenetic mechanisms, DNA methylation is the most stable (62) and remains the best understood epigenetic modification in the context of kidney disease. It describes the covalent addition of a methyl group to a cytosine residue adjacent to a guanine nucleotide (CpG dinucleotide). CpG dinucleotides are predominantly concentrated in promoter regions of genes. Hypermethylation of these sites is commonly associated with transcriptional silencing, whereas hypomethylation activates gene expression. Histones undergo posttranslational modification, including methylation, acetylation, phosphorylation, and ubiquitination that affect chromosome structure, thereby influencing accessibility of DNA to the transcriptional machinery. It is important to remember that epigenetic programming is a physiological process in normal fetal development which dictates cell differentiation; however, under adverse intrauterine conditions some epigenetic changes create marks that may become pathological.

DNA methylation and histone modification are increasingly recognized to play a role in CKD susceptibility, especially in patients with diabetes. Whereas the role of histone modification in diabetic nephropathy has been reviewed in detail elsewhere (62, 63), we will focus in this review on DNA methylation. Sapienza et al. (64) showed that gene-specific DNA methylation patterns in diabetic patients with end-stage renal disease differed from diabetic individuals without nephropa-
thy. A substantial fraction of the differentially methylated genes in this study are known to be involved in either kidney development or diabetic nephropathy. A different study utilizing renal tissue from CKD and healthy subjects showed that the epigenetic influence in CKD development was via core profibrotic pathways. Ko et al. (43) identified in genome-wide cytosine methylation assays differentially methylated loci in enhancer regions, which are enriched in consensus binding sequences for important renal transcription factors, including genes implicated in renal development and renal fibrosis. The role of epigenetic alteration in fibrogenesis was further supported by Bechtel et al. (7) who demonstrated that hypermethylation of RASAL-1, which encodes an inhibitor of the Ras oncprotein, was associated with perpetuated fibroblast activation and fibrogenesis in mouse kidneys. Methylation of CpG sites is facilitated by a group of enzymes referred to as DNA methyltransferases (DNMTs). Of the three DNMTs identified in humans, DNMT1 is the most abundant and essential in de novo and maintenance methylation. DNMT1 is induced in experimental renal fibrosis while DNMT1+/− heterozygous-deficient mice show reduced RASAL-1 methylation and are protected from renal fibrosis (7). It remains to be elucidated when the epigenetic programming in CKD occurs. While epigenetic modifications may occur in adulthood in response to environmental factors, the differential methylation of genes encoding renal development factors in Ko’s study (43) supports the notion that these epigenetic marks are established during fetal development. Thus it would provide a possible mechanistic link between fetal programming and CKD development in later life (43).

Evidence is emerging that environmental influences such as maternal smoking can alter the global and gene-specific methylation status in the offspring (14, 15, 39, 73, 74). Most of these studies use genmic DNA from placental tissue and cord blood, as well as blood and buccal cells of the offspring. An epigenetic effect of maternal smoking in offspring renal tissue is yet to be investigated. Suter et al. (74) were able to demonstrate from placenta of smoking women that among the differentially methylated genes the top canonical pathways included oxidative phosphorylation and mitochondrial dysfunction. Mitochondrial dysfunction induced by maternal smoking is therefore at least in part caused by epigenetic mechanisms. The link between epigenetic modification and mitochondrial function is also evident when mitochondrial biogenesis is assessed. DNA hypermethylation of the promoter region of the mitochondrial biogenesis regulator PGC-1α has been demonstrated in diabetic subjects, and this was inversely correlated with mitochondrial content (6, 47).

While the DNA hypermethylation of PGC-1α highlights that epigenetic modifications can influence mitochondrial function, the reverse is true as well, with mitochondria being an important determinant of epigenetic status. Recent studies in cell lines that were devoid of functional mitochondria have led to the discovery that mitochondrial dysfunction is associated with epigenetic modification of the nuclear genome (69). Smiraglia et al. (69) demonstrated that depletion of mitochondrial DNA in these cells resulted in a significant degree of aberrant CpG methylation. Methylation changes were partially reversed by repletion of wild-type mitochondria and restoration of mitochondrial DNA in these cells that were otherwise lacking the entire mitochondrial genome (69). Although other factors may play a role in DNA methylation homeostasis in human tissue, the study provides the first evidence that mitochondria may take part in regulating epigenetic modification.

**Diagnostic Implications**

The identification of mitochondrial dysfunction and epigenetic modification in CKD and fetal programming could potentially become a diagnostic tool to predict renal risk and disease progression, especially if this information can be accessed from easy to obtain peripheral blood, urine, or saliva samples. Sapienza et al. (64) used saliva to demonstrate a difference in gene-specific DNA methylation levels in a renal disease cohort vs. control. Although there is a common perception that an individual’s epigenetic DNA methylation profile may differ between tissues, two studies have shown that the majority of CpG sites are methylated similarly between different tissues. Rakyan et al. (60) estimated that only 18% of the ~25,000 genes analyzed in 16 different tissues showed a tissue-specific methylation pattern. Sapienza et al. (64) corroborated this result by showing 11% variation in methylation patterns of >50,000 CpG sites comparing human cord blood and placenta samples. Further studies are needed to confirm that DNA methylation profiles derived from blood cells or even saliva could be used as a viable surrogate for localized organ-specific disease such as CKD. Furthermore, confirmation in prospective studies are required to ensure that global or gene-specific DNA methylation as a biomarker is not influenced by the disease process or treatments associated with the disease, i.e., dialysis. While the evidence for a correlation between adverse environmental factors in early life and DNA methylation causing disease susceptibility is emerging, it also needs to be replicated in larger longitudinal studies.

**Therapeutic Targeting**

The other key area where epigenetics may have increasing relevance in nephrology are epigenetic-based therapies that inhibit DNA methylation. In the field of hematology, targeting of epigenetic mechanisms is already integrated in treatment regimens. The demethylating agent 5′-azacytidine has been approved for clinical subtypes of myelodysplastic syndrome and acute myeloid leukemia, conditions in which aberrant CpG methylation plays a role. As aberrant hypermethylation appears to be implicated in renal fibrogenesis, erasing the pathological methylation may provide a useful therapeutic concept. 5′-Azacytidine has had an antifibrotic effect in vitro in human kidney fibroblasts as well as in mouse models of renal fibrosis (7). However, 5′azacytidine, which is incorporated into DNA, results in genome-wide demethylation and has substantial cytotoxicity. Hence a more target-specific approach may be needed.

Targeting mitochondrial dysfunction as a therapeutic strategy in renal disease has also gained considerable interest. Strategies focus on prevention of mitochondrial injury by mitochondria-targeting antioxidants, acceleration of mitophagy, a cellular process that helps to degrade injured mitochondria, and replacing dysfunctional mitochondria through stimulation of mitochondrial biogenesis. Antioxidants designed to accumulate in mitochondria have been developed. The most extensively studied of these mitochondria-
Cigarette smoke exposure leads to Placental vasoconstriction and morphological changes, which results in Low birth weight. Low birth weight contributes to Type 2 Diabetes, Obesity, CV disease HTN, Impaired nephrogenesis, Reduced nephron number, glomerular hyperfiltration, compensatory hypertrophy, Glomerulosclerosis, which eventually leads to CKD.

In fetal life, aberrant fetal DNA methylation results in Fetal mitochondrial dysfunction, which is associated with PGC-1α downregulation. PGC-1α is a key regulator of mitochondrial biogenesis and oxidative phosphorylation. Its downregulation in fetal life can lead to compromised mitochondrial function and energy metabolism, predisposing to chronic diseases in adult life.

In adult life, CKD can progress to ESRD, requiring dialysis or transplantation. The burden of kidney disease is significant, with an estimated 850,000 new cases of CKD diagnosed in the United States each year. Therefore, interventions to prevent or delay CKD progression are crucial.
References


