The role of epigenetics in renal ageing.

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Abstract
The proportion of elderly in the World's population is growing, bringing with it an increase in age related morbidities. An ability to understand and separate natural aging processes from processes specific to morbidities is therefore required to understand the heterogeneity observed in the processes of age related organ dysfunction. Mechanistic insight into how epigenetic factors regulate aging throughout the life course, linked to a decline in renal function with aging, is already proving of significant value. Non-coding RNAs have been shown to provide epigenetic regulatory circuits within the kidney, reciprocally interacting with DNA methylation processes, histone modification, and chromatin. These interactions have been demonstrated to reflect the biological age and function of renal allografts. Significantly, these epigenetic factors control gene expression and activity in response to environmental perturbation and significantly are also key and highly conserved signaling pathways known to modulate aging, including the mTOR and the insulin/IGF signaling pathways, and the sirtuin family.
Potential mechanistic links between the epigenetic landscape of aging and renal dysfunction have emerged centering on nutrition (phosphate/ phosphatidylcholine), gut microbiota and inflammation. These are directly affected by environmental factors, including psychosocial and lifestyle stresses.
Modification of the epigenome in the kidney via nutritional interventions, or targeting the methylome, or chromatin, appear eminently feasible, though caution is merited as intergenerational or transgenerational effects in man are not always obvious.

Key Points:

- Deaths due to renal dysfunction are growing globally, despite other etiologies showing significant decline in the midst of an increasingly aging world population.
- A mechanistic link is emerging between the epigenetic landscape of aging and renal dysfunction based on regulation of aging processes that are common across taxa.
- Epigenetic regulation of biological ageing in the kidney is influenced directly by nutrition, inflammation, the gut microbiome, psychosocial and lifestyle factors.
- Models of progeria provide important hypothesis-generating insights for the future therapies designed to modify the epigenetic landscape in ageing and disease.

These interactions indicate new avenues for epigenetic interventions to improve renal health, though caution is merited as intergenerational or transgenerational effects in man are not always obvious.

An introduction to aging in the kidney

Geroscience, champions modifiable aspects of biological aging with a view to enabling healthier old age. It focuses on common mechanisms of aging, as knowledge of the mechanisms and modification of the aging process would be expected to affect a broad range of age-related morbidities[1,2]

This is in keeping with a major shift in focus by the World Health Organization (WHO) in response to the changing demographics of human society and an anticipated major burden of age related morbidity and associated societal health care costs. By 2020, the number of people aged 60 years and older will outnumber children under 5 years old. By 2050, those over 60 years will outnumber those below 14 years and constitute 2 billion of the world's population. Consequently, the WHO places increased emphasis on promoting better age related functional health.

When evaluated in the context of renal dysfunction, these projections mask the impact of age related decline in renal health. Healthy life expectancy (HALE) and disability-adjusted life-years (DALYs) data, have indicated deaths due to chronic kidney disease (CKD) and some cancers have increased globally despite other etiologies showing significant declines in age standardized DALY rates amid rising total DALYs[3].

In this respect, allostatic load can be defined as a composite indicator of accumulated biological stress through the life course, which predisposes to morbidity in the face of chronic or repeated stress exposure.[6] Assessing allostatic load is not straightforward and necessitates an integrative analysis of a series of biomarkers for stress, aging, and immune function.[6]

Additionally, it needs an understanding of epigenetic effects that are differentially responsive to environmental changes at different stages in the life course and which can be transmitted across generations. These mediate interplay with a range of environmental factors, namely psychosocial, socio-economic, nutritional and lifestyle factors, that affect health span. [7,8] These are often neglected, or overlooked, in favor of mechanistic approaches to understanding renal aging and pathology.

Aging and renal dysfunction

Distinguishing normal features of the aging process from age-related dysfunction is critical to understanding age-related renal health. This is pertinent to a range of morbidities, [1] but in the first instance can be exemplified in the context of CKD. The incidence of CKD is ~10-12% in the developed world and growing.[9] Notably, the incidence of CKD parallels chronological aging and almost 50% of end stage renal disease (ESRD) patients are 65 years or older. There seems to be heterogeneity in the processes that lead to CKD and factors beyond chronological age, such as epigenetic alterations are likely to contribute to a decline in renal functions during aging.[1]

Accelerated cellular and physiological aging, however, are already established underlying components of a number of morbidities, including CKD.[1,10–12] Correspondingly, CKD patients show a higher incidence of mortality in comparison to healthy chronologically age matched individuals, indicative of systemic differences layered on top of the dysregulated aging process (Figure 1). As the prevalence of CKD parallels an increased prevalence in type-2 diabetes, obesity and a sedentary life style,[13], an allostatic outcome reflecting the ”burden of life style” may be present next to effects of CKD *per se*. As life style factors directly affect the epigenome,[14] the effects of epigenetic changes on premature biological aging on renal dysfunction merit interest.

Loss of plasticity and/or resilience to adaptions to the changing internal uremic environment promote vascular aging processes and result in a marked discrepancy between the chronological and biological age in CKD.[1] As the kidney ages chronologically, it displays a range of functional and structural changes commensurate with a decrease in glomerular filtration rate, altered sodium regulation, increased renin-angiotensin activity, nephron loss, tubular atrophy and diverticula formation and decreased tubular number, interstitial fibrosis and basement membrane thickening. Parenchymal calcification and renal arterial atherosclerosis are also observed.[1,15] The uremic phenotype is characterized by many features that typically characterize the aged population, such as frailty, depression, cognitive dysfunction, sexual dysfunctions, hypogonadism, inflammation, gut dysbiosis, protein-energy wasting (PEW), osteoporosis and premature vascular aging, including both

atherosclerois (i.e. media thickening) and arteriosclerosis (i.e. media calcification).[1,16,17]

Aging in mammals brings an accumulation of senescent cells within the body, associated with a loss of functional capacity and characterized by $p16^{ink4a}$ expression accompanying cellular growth arrest and a distinct epigenotype.[15,18] Change in NF-κB expression is also typical, with subsequent production of a pro-inflammatory senescence associated secretory phenoytpe (SASP) that enables paracrine mediated signaling to adjacent tissue (i.e. bystander effects), which may spread allostatic load via secretion of SASP factors and extracellular secretory vesicles via the circulation.[19,20] Knock-on effects include loss of regenerative capacity and a decrease in the availability of nitric oxide.[21] As the cardiovascular system is vulnerable to the uremic milieu, CKD represents a segmental progeric syndrome.[22] Understanding the nature of premature aging mechanisms in the perturbed uremic milieu may therefore be a prerogative for the rational development of prophylactic measures, as well as novel and targeted treatment strategies for renal dysfunction (Figure 1).

Biomarkers of aging in man

Increasing chronological age (i.e. years since birth) results in a loss of physical capability and function, but the degree of inter-individual variation in people of the same chronological age is substantial and adumbrated by a host of environmental, genetic and epigenetic contributors. There is thus no 'gold standard' for determining what constitutes normal aging. Biomarkers of aging (BoA)-related measures of health related function should therefore show a significant association with chronological age, specifically in the absence of disease[23,24] Over the past 50 years a number of candidate BoAs have been developed, but all have proven insufficiently robust. Systematic reviews of the evidence relating telomere length to mortality/life span and to normal age related decline in health measures found few actual mortality studies, all of which suffered from survivor bias.[7,25–27] Few studies have examined the relationship with age-related functional decline, and those that have were equivocal and all lacked statistical power.[8,28]

Longitudinal analyses of renal allografts, acting as a source of healthy tissue and yielding eGFR as a functional measure of health, have already supported the use of CDKN2A expression as a validated biomarker of aging.[10,29,30] This has led to the

identification of a small miRNA based epigenetic signature associated with regulation of the CDKN2 locus that similarly fulfils this criterion and importantly, which is active in aging related biochemical pathways, including mTOR and PI3K-AKT, conserved across taxa.[22] Additionally, these also help regulate chromatin modifiers, notably sirtuin family members, thus linking nutrient sensing, cellular metabolism and stress defences to age related renal function.[31] As such, the activities of these miRNAs fall within the nine hallmarks of aging described by Lopez Otin et al (2013)[25] that represent common denominators of aging in different organisms. Additionally, a number are in turn epigenetically regulated by DNA methylation, in keeping with the activities of a methylation clock.[26] These will be discussed in more detail below.

What is epigenetics?

The term epigenetic denotes heritable changes in gene expression that do not involve changes to the underlying DNA sequence (i.e. a change in phenotype without a change in genotype). Epigenetic regulation facilitates biological plasticity in response to environmental changes and enables transgenerational transmission of such responses. Addressing age-related changes in epigenetic plasticity is, however, challenging, as changes in chromatin, metabolic networks and tissue structure and function, appear to underpin a range of age related diseases.[34,35]

Two distinct forms of epigenetic inheritance can be described comprising those engendering an intergenerational effect and those engendering a transgenerational effect. An intergenerational epigenetic effect can be induced during development by the intrauterine environment, thus transmitting them from one generation to the next. Transgenerational epigenetic inheritance occurs when developmental programming can be transmitted across generations not subject to the initial environment stimulus that triggered the epigenetic change.[36] These are illustrated in Figure 2. Little is known on their impact on kidney disease, but a role for intergenerational effects is intuitive[1].

Methylation

Classically, a loss of genomic methylation and associated heterochromatin has been considered features of the aging cellular genome.[37] Genomic methylation levels display age related epigenetic drift which may directly affect aging processes.[38] Age related changes in genomic DNA methylation (gDNAm) patterns have also been

formulated as an epigenetic clock. These show excellent correlation with chronological age[33,39,40], though strong correlations with biological age (i.e. age related biological function) remain to be proven. Despite this, there is clear evidence that changes in the methylome incorporated into epigenetic clocks are predictive of all-cause mortality even after adjusting for chronological age and a variety of known risk factors [41–43]

A methylome based epigenetic age has been clearly associated with longevity, frailty and cognitive/physical fitness. It has been used to demonstrate accelerated ageing in a range of conditions including diverse neurodegenerative conditions (Parkinson's disease, Alzheimer's disease-related neuropathologies), lung cancer, lifetime stress, menopausal age, obesity, metabolic syndrome and coronary heart disease (CHD).[44] Furthermore, it has been shown to reflect accelerated ageing associated with environmental stressors, such as poor diet, lack of exercise and chronic low grade inflammation[45]

More recently, mitochondrial DNA methylation (mtDNAm) at two CpG sites (M1215 and M1313) located within the 12S ribosomal RNA gene has been demonstrated to correlate inversely with chronological age and thus may provide additional epigenetic markers of aging and function, when combined with gDNAm markers.[32] As a number of genome wide association studies have not found a correlation between gDNAm and altered age related gene expression,[47,48] the incorporation of mtDNAm might help remove any equivocation in associations with age related functional parameters of health.

A number of clinical observations and rodent model studies have indicated the changes in the methylome are implicit in the development and progression of CKD and are elegantly summarized by Ko and Susztak.[49] How any such changes relate to and , or, interplay with the aging process *per se*, remains to be determined. However, such changes do appear to be associated with renal pathology in CKD.[50] Notably, they provide a platform for the development of metabolic memory, particularly in the context of diabetic nephropathy.[51,52]

Chromatin structure

Chromatin forms through packing of DNA around nucleosomes in a number of distinct three dimensional tiers, enabling assembly of higher order structures that interact directly with the nuclear envelope and that link otherwise distal genomic regions.[18,53] Chromatin is transcriptionally inactive and its structure changes with age, typified by loss of bound heterochromatin protein 1 (HP1) and H3K9me3 marks.[54] These losses are also observed in human progeric syndromes, such as Hutchinson Guilford Progeria Syndrome (HGPS) [57,58] and Werner Syndrome (WS.). [59]

Non-coding RNAs

Critical mediators of epigenetic plasticity within the cell, and indeed the organism, are non coding RNAs (ncRNAs), which effect and co-ordinate regulation of a complex range of molecular processes in response to environmental changes. The aging process is dramatically influenced by ncRNA activity..[60–62] A number of distinct classes of ncRNA have been identified, mainly comprising micro (miRNA) and long non-coding (lncRNA) RNAs. These provide epigenetic regulatory circuits in which lncRNAs, miRNAs and their target transcripts, reciprocally regulate expression and include regulatory interaction with DNAm, histone modification, DNA methyl transferases (DNMTs) and chromatin, reciprocally controlling their expression and activity. [63]

Long non-coding RNAs (lncRNAs)

LncRNAs constitute non-coding RNAs over 200 nt long, which regulate gene expression patterns at the transcriptional, post-transcriptional and post-translational levels. LncRNAs display tissue specific gene expression[64,65] and are thought to have direct roles in the aging process. Putative roles for other lncRNAs have been identified in conditions such as heart failure, cardiac autophagy, hypertension, acute kidney injury, glomerular diseases, acute allograft rejection and renal cell carcinoma. [65] LncTERC and lncTERRA are well described in their capacity to regulate telomeres. [62] Congruent with miRNA regulation of cellular stress and metabolism in aging, regulation of Igf2, which encodes an insulin-like growth factor involves lncRNA H19 regulation of MBD1 (a methyl-CpG-binding domain protein). [66,67,68] Interestingly, H19 expression is altered in the kidneys of embryos carried by hyperglycemic mothers, providing a conduit for an *in utero* transgenerational transmission of predisposition to related renal dysfunction (Figure 2). Pertinent to renal dysfunction

lncRNA Tug1 has been demonstrated to connect metabolic changes with kidney disease in podocytes. In particular, it links mitochondrial bioenergetics, which show age related dysfunction in a wide range of species, with nephropathy.[69]

More directly adumbrated by aging is CDKN2B-AS/ ANRIL (antisense non-coding RNA in the INK4 locus). [70] ANRIL is thought to function as a transcriptional repressor through interaction with CBX7 and SUZ12, components of polycomb repression complexes 1 and 2 respectively, which modify local chromatin status. [71,72] In the instance of CBX7, ANRIL facilitates recruitment to the CDKN2A locus and resultant increase in H3K27methylation, which epigenetically represses CDKN2A ($p16^{ink4a}$) transcription. [71,73]

microRNAs

MicroRNAs (miRNAs) comprise an evolutionary conserved group of ubiquitously expressed single-stranded 20-24nt entities that act as negative post-transcriptional regulators, either via RNA silencing or translational inhibition. [74,75] They can show tissue specific expression and are subject to epigenetic regulation by differential methylation and chromatin changes. At present 1881 functional miRNAs have been characterized for the human microtranscriptome, which are thought to target ~ 60% of protein coding genes in man. [76,77] However, over 4000 putative candidates have been identified by sequence searches. [78]

Isoforms (isomiRNAs) of archetypal miRNAs (reference sequence listed in miRNA databases), with different 5 prime and/ or 3 prime termini generated from the same pre-miRNA have also been identified. Specific isomiRs have been reported as substantially more abundant than the archetype miRNA. [79] The available knowledge on isomiRNA targets, however, functions and involvement in disease processes remains sparse.

Conversely, knowledge of archetype miRNAs and their role in the kidney is abundant. These have been implicated in renal physiology, development and pathology. [65,80–84]

The interactions of miRNA identified as important for age related renal function indicates they facilitate the maintenance of an intracellular regulatory network consistent with the original mitochondrion/telomere nucleoprotein complex/ribosome (MTR) postulate of Shiels and Davies (2004)[85], where intracellular

energy availability determines a cell's capacity for proteosynthesis in response to macromolecular damage/redox stress and nutrient availability and utilization via mTOR. [85,86] This is in keeping with positive results from interventions promoting health span and longevity, such as calorie restriction and administration of rapamycin, which work directly through mTOR. [87,88]

Epigenetics and progeria

To better understand how the epigenetics of aging impacts on the renal biology, it can be instructive to examine the pathology of accelerated aging syndromes with elements of renal dysfunction. In this respect, Hutchinson-Gilford progeria syndrome (HGPS) provides value. HGPS is one progeroid syndrome that recapitulates cellular and molecular characteristics of physiological aging and show wide changes in the epigenetic landscape.

HGPS is a monogenetic disorder caused by mutations in the *LMNA* gene. [89,90] It shares along with physiological aging both cellular (abnormal nuclear morphology, global genomic hypomethylation and increased DNA damage) and tissue pathology (loss of subcutaneous fat, loss of hair, reduced bone mineral density and CVD). [91] Accumulation of progerin in HGPS is marked by alterations in chromatin structure, including a progressive loss of heterochromatin, perturbation of the methylome and impaired genome stability. [92–94] Interestingly, low levels of progerin have been found in skin and arteries from non-progeria individuals with increasing levels during aging. [95,96] Progerin expression is also induced by senescence and telomere damage, [97] both features of renal aging and dysfunction[98] Up-regulation of senescence associated secretory phenotype (SASP) factors and increased senescence-associated β-galactosidase staining frequency have been shown in HGPS mouse models. [99] Correspondingly, activation of NF-κB signaling in this context also provides a link between nuclear lamina defects and systemic inflammation; a key feature of both the progeric and uremic phenotypes. [100,101] The vascular phenotype of uremia also exhibits the calcification and increased arterial stiffness characteristic of HGPS. One direct sequitur from this hypothesis is that other non-communicable diseases may generate similar aging effects. This merits further investigation and testing. An additional feature of HGPS is elevated hyperphosphatemia, indicative of diminished

renal function. Whereas hyperphosphatemia is a classical complication of CKD, it has also been observed in and in general population cohorts, linked directly to nutrition[1]

Analogous to HGPS in many respects is Klotho, mutations in which generate progeria with concomitant hyperphosphatemia. Klotho is critical to modulating the function of FGF23, a phosphaturic and calcium and sodium-conserving hormone, as part of the maintenance of phosphate and vitamin D homeostasis, in concert with parathyroid hormone (PTH).[102] FGF23 is also active in bone mineralization and has been implicated in the impairment of cardiac volume homeostasis in CKD. Both FGF23 and PTH levels are chronically raised due to phosphate retention and decreased renal 1,25(OH)2D3 production, resulting in vascular calcification. [103–106]

Klotho-deficient mice display extensive features of progeria,[84] including infertility, vascular calcification, atherosclerosis, organ atrophy, osteoporosis, peripheral insulin sensitivity, metabolic dysfunction and cognitive changes. Polymorphisms in the Klotho gene have been associated with human aging and health span. [108]

Recently, the anti-aging properties of Klotho in endothelial cells were explained by inhibition of NF-κB translocation from cytoplasm to the nucleus, via stabilization of the NF-κB/IKK complex, which protected these cells from senescence. [109] There appears to be a strong epigenetic regulation of Klotho expression in uremia. Uremic toxins such as indoxyl sulphate induce hypermethylation of the Klotho gene[110], possibly mediated through oxidative stress and induction of pro-inflammatory transcription factors such as NF-κB.[111] Therefore, a reduction in Klotho expression may not only result in cellular senescence, but may also inhibit an important negative feedback loop resulting in increased inflammation.

Epigenetics and inflammaging

An important determinant in the pathogenesis of premature aging in range of morbidities at different system levels (from (epi)genetics to the macroscopic phenotype) appears to be the presence of inflammation.[112] In this respect, there appear to be relevant correlates between inflammation in renal dysfunction and aging and

“inflammaging” in the general population.[1,113,114] The term "inflammaging." has been used to describe chronic, low-grade inflammation, which is a proven risk factor for morbidity and mortality in the aged. Many age-related morbidities present with an underlying component of low-grade inflammation, though its etiology remains undetermined.[115]

Both DNA hypermethylation, and telomere shortening linked to persistent inflammation have been observed in independent studies on CKD.[98] The former observation, however, is counter-intuitive, as DNA hypomethylation is more typical of accelerated aging. [45] Observations of hypermethylation in CKD thus merit further study, as they may reflect a distinct epigenetic feature of CKD pathology decoupled from natural aging processes.

We have previously indicated associations between inflammatory biomarkers on DNA methylation in CKD.[113] Inflammation directly affects the activity of enzymes involved in the establishment, maintenance and change of epigenetic landscape across the lifecourse.[116,117] As DNMTs are directly affected by IL-6, changes in methylation dynamics can be predicted to lead or predispose to subsequent ill health.

One example of renal dysfunction linked to methylation status involves homocysteine. In CKD, circulating homocysteine levels are elevated, enabling its conversion to S-adenosylhomocysteine (SAH). SAH is a competitive inhibitor of methyltransferases and thus has a direct effect on the cellular methylome.[118] Recent evidence has indicated a link between immunosuppressive therapy, aging and homocysteine biochemistry in renal transplant recipients.[119] Longitudinal analyses of aging in CKD have indicated that these patients displayed significantly advanced biological age.[98] Notably, homocysteine is critical for DNA methylation nucleotide biosynthesis and maintenance of genomic integrity.[120,121] Impact on the cellular methylome in these circumstances seems intuitive, but requires further investigation to determine if any changes in its status either drive the accelerated aging, or are a consequence of it, or of any clinical interventions.[119]

At a cellular level, recent evidence suggests an important role for the SASP in the pathogenesis of inflammaging. Senescent cells are pro-inflammatory [122].[123] and generation of a SASP appears to be under strong epigenetic control. For example, the

histone deacetylase Sirtuin 1 may prevent gene expression of pro-inflammatory transcription factors, such as NF-kB as well as transcription of pro-inflammatory cytokines, such as IL-6, which play an important role in the conversion to a SASP producing cytotype.[124] In addition, the Jumonji D3 histone demethylase can induce both inflammation as well as senescence.[125] These findings are of potential significance, as Sirtuin 1 is amendable to pharmacological interventions (e.g. by resveratrol), whereas JMJD3/KDM6B can be selectively inhibited by GSK-J4.[126] However, although resveratrol reduced vascular calcification in uremic mice and improved aerobic capacity compared to controls,[127] the relevance of these interventions for human CKD have not been studied yet.

Environmental drivers of epigenetic changes and age related health

The questions remain as to exactly (i) how factors in an organism's external environment lead to the molecular pathophysiological changes associated with aging and disease? (ii) How such factors may induce heritable transgenerational physiological changes? There is now a wealth of information emerging on psychosocial drivers of age related health, principally indicating that the early years of the life course as a key period for establishment of their effects. [7,128,129] This paradigm is encapsulated within the Developmental Origins of Health and Disease.[130] As such, stressors, both early life and subsequently through the life-course, acting cumulatively, interactively or independently, may lead to individual differences in health span. Conceptually, allostatic load can therefore integrate a number of overlapping bio-psychosocial frameworks that have been formulated to explain these differences. [131,132] A full discussion of these is beyond the scope of this review, but is elegantly covered by Rubin[6]

We have also hypothesized that *how and when* any pathology manifests in the kidney is influenced by inter-individual differences in a range of factors (see Figure 1 and Figure 3) impacting on the epigenetic landscape. These include genetics, lifestyle factors, psychosocial factors and nutrition.[133] In particular, it is intuitive that such inter-individual differences will impact on organ dysfunction and associated co-morbidities, progression rate and both timing and severity of associated co-morbidities. Although recently a GWAS study showed a relation between CKD progression and SNPs in *LINC00923*, an

RNA gene expressed in the kidney, research into the relation between individual risk factors and epigenetic alterations relevant for kidney disease is limited[134] However, a review of recent literature presents a consistent picture on how these might influence the epigenetic landscape of aging and health span.[135] Significantly, a meta analysis of early life adversity and biological ageing (determined by telomere length) also supports the thesis that early adversity has long-lasting physiological consequences contributing to disease risk and biological aging and hence diseases of ageing.[136] Notably, both the type and timing of early adversity significantly impacted the association with telomere length. This has been paralleled by studies in rodents, which have indicated that intrauterine exposure to maternal stress alters telomere length in the brain of adult offspring.[137]

While studies in model organisms[37] have indicated that nutrition has transgenerational epigenetic effects, in man the majority of evidence comes from two cohort studies. These comprise the 'Dutch birth famine' and the Överkalix studies, which have been reviewed in detail elsewhere.[133] Evidence from the Dutch birth famine cohort found trans-generational transmission of effects, including increased neonatal adiposity and type 2 diabetes and cardiovascular disease.[138,139] One critical observation in this context, was that hypomethylation of the imprinted IGF2 gene was associated with prenatal famine and contributory to their associated insulin resistance.[140,141]. Interestingly, IGF2 transcription is regulated by miRNAs implicated in the control of age-related renal function.[29] Notably, miRNAs 125a-5p, 125b, 96 and 217, which have been shown to be associated with age-related renal health span, all regulate sirtuin targets. As such, the expression levels of these biomarkers may provide a platform for a molecular quantification of allostatic load linked to physiological function at a given point in the life course.

Inter-individual differences in genomic methylation have also been observed in relation to methyl-donor nutrient intake by mothers of children born during periods of good food availability or poor food availability. Children born during a time of poor food availability, corresponding to higher maternal methyl-donor nutrient intake peri-conception, displayed increased methylation

of six metastable alleles.[142] Methyl donor availability is also directly pertinent to renal allograft function and biological aging, as is discussed above.[98] Additionally, genomic hypomethylation, along with telomere attrition, both features of advanced biological aging, have been observed in socioeconomically deprived individuals in the general population.[143,144] This correlated with chronic inflammation in such communities and a greater burden of morbidity and earlier mortality. Notably, recent evidence has indicated that this is also independently associated with nutritionally-driven hyperphosphatemia, due to frequent red meat and processed meat consumption.[145] These findings are also relevant to the growing incidence of CKD globally and indicate that nutritionally driven hyperphosphatemia associated with lower socioeconomic status and poor eating habits are potential contributory factors to CKD and chronic inflammation.[145]

The question then arises how nutritionally acquired phosphate affects the epigenome? Direct evidence for this in man remains sparse. Recent evidence, however, suggests that inorganic phosphate induces aberrant promoter methylation in Endothelial-Mesenchymal Transition, via altered DNMT1–HDAC2 interaction in human coronary endothelial cells, which might contribute to endothelial dysfunction in vivo.[146]

Aging, epigenetics and the microbiome

Although a discussion on aging and inflammation is incomplete without mentioning the microbiome, the effect of the gut microbiota on renal function is beyond the scope of this article and has been covered in depth elsewhere.[147] However, central to the inter-relationship between gut microbiota, inflammation and the age related renal epigenome is Trimethylamine N-oxide (TMAO), a metabolite derived from microbial metabolism, which is found in elevated levels in advanced CKD where it is an independent predictor of mortality. [148] TMAO is produced from the metabolism of phosphatidylcholine, L-carnitine and lecithin, found in red meat, fish and eggs. It is a pro-atherogenic and pro-inflammatory compound that provides a mechanistic link between excess red meat consumption in man linked to both hyperphosphatemia and accelerated aging. [145,149,150] Notably in these studies, less than 25% of inter-individual variation in inflammatory status was

explainable by biological age, [143] or methylation levels, [151] which suggest that the microbiome might have a larger impact on inflammation than anticipated based on a pre-existing hypothesis of a linear relationship between biological age and SASP related inflammatory status.

Mechanistically the cumulative and interactive effects of different processes may be at play here. Firstly, nutritionally derived hyperphosphatemia with concomitant tubular injury and interstitial fibrosis due to calciprotein particle (CPP) generation, and genomic hypomethylation, occurring independently of telomere attrition.[145] Unsurprisingly, the odds ratio of developing CKD is higher in healthy subjects with a serum Pi >4mg/dL[152] and a high red meat intake[153] Additionally, translocation of gut microbiota (and, or endotoxins) into the circulation via disruption, or leakage, at colonic epithelial tight junctions through altered occludin and claudin expression might also be at play. [154,155] Dysfunction of tight junction proteins has also been reported to result in reduced nuclear factor erythroid 2-related factor 2 (Nrf2) expression, thus elevating oxidative stress levels.[89,129] Elevated microbial endotoxin levels have previously been detected in the circulation of uremic patients and implicated in generating a pro-inflammatory environment.[156] The upshot of such processes is an increase in inflammation that has a direct affect on DNMTs and histone modifiers, with consequential effects on the epigenome. A direct implication of this is that the type and caliber of nutrition across the life course may impact significantly on the accrual of allostatic load. Further investigation of these processes in the uremic environment is warranted.

Modifying the epigenome in aging

Although this field remains immature within regard to nephrology, a range of evidence from model organisms through to man, indicates that nutrition, exercise and psychosocial interventions can mitigate the effects of aging.[157–160] What is encouraging, is that such interventions target pathways that have already been demonstrated to be important in the epigenetic regulation of age-related renal function[1,22] and include signaling and the nutrient-sensing kinase AMPK and mTOR pathways,. The clinical agents metformin and rapamycin respectively target these pathways, slowing cellular aging and reducing pro-inflammatory SASP generation. [161–163] Bromodomain-containing protein 4

(BRD4), which binds to acetylated lysines on histones, is also able to inhibit generation of a SASP in murine models, but this comes at the expense of impairing immune surveillance[164]

Modulation of sirtuin biochemistry and insulin/IGF signaling has also been demonstrated to have an impact on organismal health span.[18,87,160,162,163] Sirtuins comprise orthologues of yeast Sir2.[161] In man, there are seven sirtuins which span the intracellular environment across the components of the MTR[60,86,161]. All seven require NAD+ as an obligatory cofactor for their activity, though their respective roles differ based on their subcellular localization, substrate specificity and spatio-temporal expression. They constitute deacetylase, deacylase, desuccinylase, demalonylase, deglutarylase and ADP-ribosyltransferase activities that enable dynamic cellular responses to metabolic, redox and circadian changes, [165] that fall within the hallmarks of aging described by Lopez Otin et al. [32]

The loss/decline in sirtuin activities with age may facilitate age-associated changes in the epigenome and associated transcriptional profiles. Animal studies have indicated that loss of function for individual sirtuin family members can have dramatic effects on chromatin regulation and lead to progeria and a diverse range of age associated pathologies.[86] The renal aging miRNAs 125a-5p, 125b, 96 and 217, all regulate sirtuin targets. These miRNAs are also active in the mTOR and PI3K pathways, which along with the sirtuins, are involved in regulation of inflammation, cellular stress responses, autophagy in response to caloric restriction and exercise.[166] Consistent with such a thesis, CR-induced epigenetic changes have also been demonstrated to ameliorate age-dependent aberrant methylation in rodent kidneys indicative of improved health span.[167]

Targeting the cellular methylome also holds promise and a range of chromatin remodeling agents are in development for both pre-clinical and clinical testing. A number of studies in model organisms have already indicated a functional relationship between differential DNA methylation, transcriptomic effects and the development of age-related renal pathology.[139,140] This is of particular interest, as methylation-based epigenetic changes in CKD models have been linked with high serum phosphate,[141,168] so it is intuitive that interventions including synergistic removal of calciprotein particles, might also have some effect in mitigating any adverse effects.

Pertinent in the context of uremia is modulation of Klotho methylation, possibly in combination with dietary interventions to reduce phosphate intake. Klotho promoter methylation is a feature of CKD, but treatment with Rhein (an anthraquinone compound used to treat diabetic nephropathy) reverses klotho methylation in murine models.[87,88] Rhein exhibits various renoprotective functions and effectively corrects DNMT1/DNMT3a induced klotho hypermethylation.[169]

Conclusions

The epigenetics of aging is clearly important for health span and is likely context dependent Consequently, approaches to remodel the epigenome need to be investigated across the life course, to determine if their effects are uniform, or even reversible, over time, or if they demonstrate effects which fall under the aegis of antagonistic pleiotropy. Cellular senescence, a key component of organ and tissue aging, appears to fall within this context, being undesirable as we gain biological capital early in the life course, but desirable later in the life course to prevent cancers. It is thus intuitive that its associated epigenetics might also be subject to context dependent intervention. This might be a contributory factor to attempts to functionally reprogram cells from older individuals, which despite having a rejuvenated transcriptional profile retain some age-related characteristics.[170]

How one reconciles changes observed in epigenetic regulation of aging processes across the body with differences due to segmental aging and, or, tissue or organ specific disease processes, remains to be resolved. Caution must therefore be used in interpretation of any initial successes with these agents, especially when directly extrapolating information derived from model organisms to clinical use in man. The transgenerational effects resulting from the use of the endocrine disruptor vinclozolin, for example, did not emerge until F1–F4 generations, when animals were found to be associated with altered epigenetic programming of the male germ line and display of kidney disease, immune system dysfunction, prostate disease, hypercholesterolemia and an increased rate of cancer.[171]

Additional approaches targeting modification of the epigenetic landscape include cellular reprogramming via miRNA delivery to enhance renal repair, which is also

showing promise for use in the clinic,[18,161] though it remains pre-clinical at present. Additionally, senotherapeutics, especially promising agents that can remove aged cells, are also in development and preclinical testing, but their impact on the epigenome remains to be established.[172] The potential of this approach, however, has been elegantly demonstrated recently, with the selective removal of senescent cells by apoptosis using a FOXO4 peptide, which restores tissue homeostasis and renal function in naturally aged mice.[173]

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Figure 1. The epigenetic landscape mediates the interplay between stressors and renal dysfunction

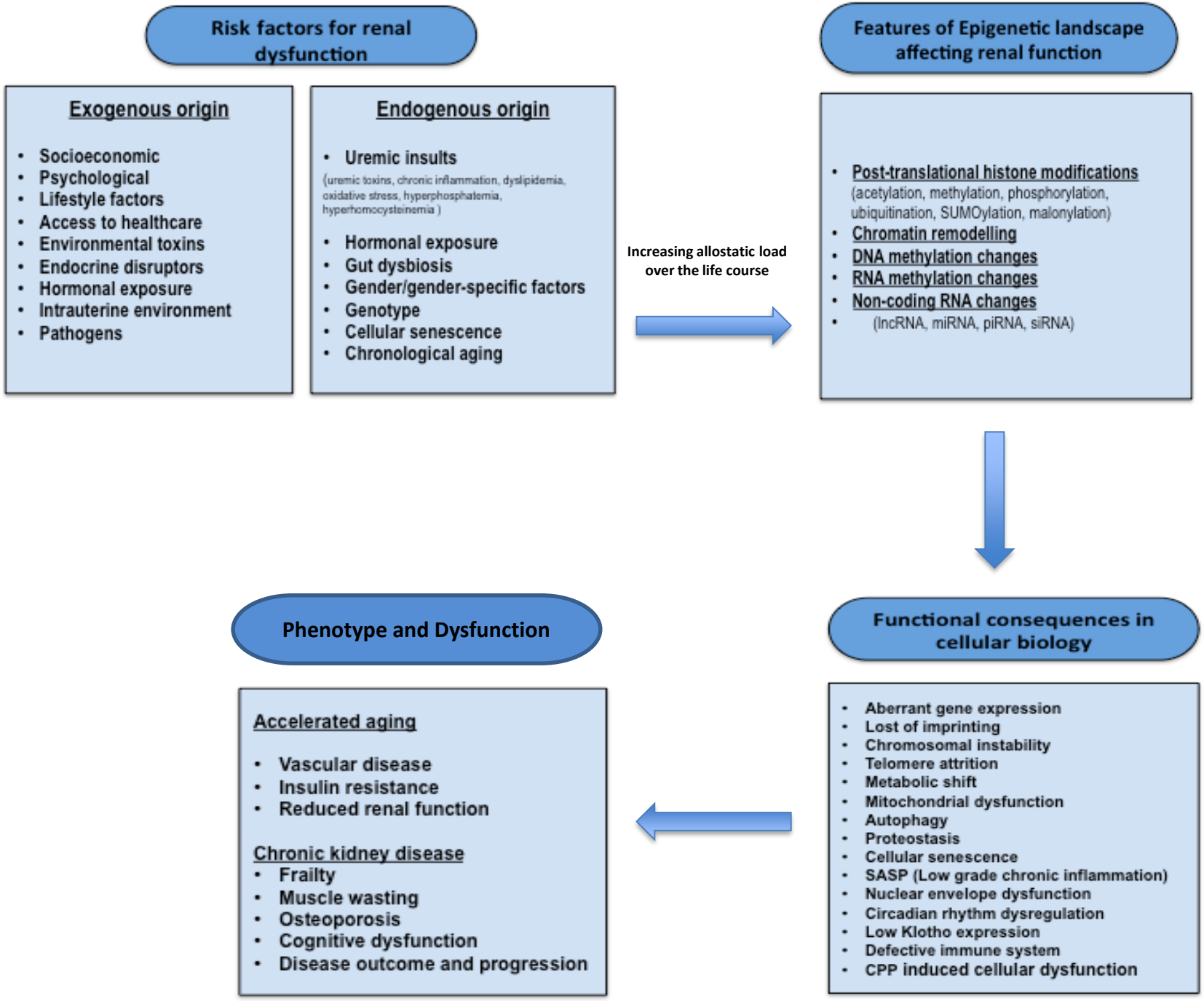
Both exogenous (environmental) and endogenous (physiological) stimuli lead to changes in the epigenetic landscape that impact on renal aging and physiology. These reflect accumulating allostatic load, both physiologically across the body and at the cellular and molecular levels. The resulting functional changes in cellular biology, that reflect accelerated aging processes in the organ, act in concert with stress driven physiological changes to induce further dysfunction that predisposes to and aids progression of disease processes, which may further perturb all the tiers of the epigenome.

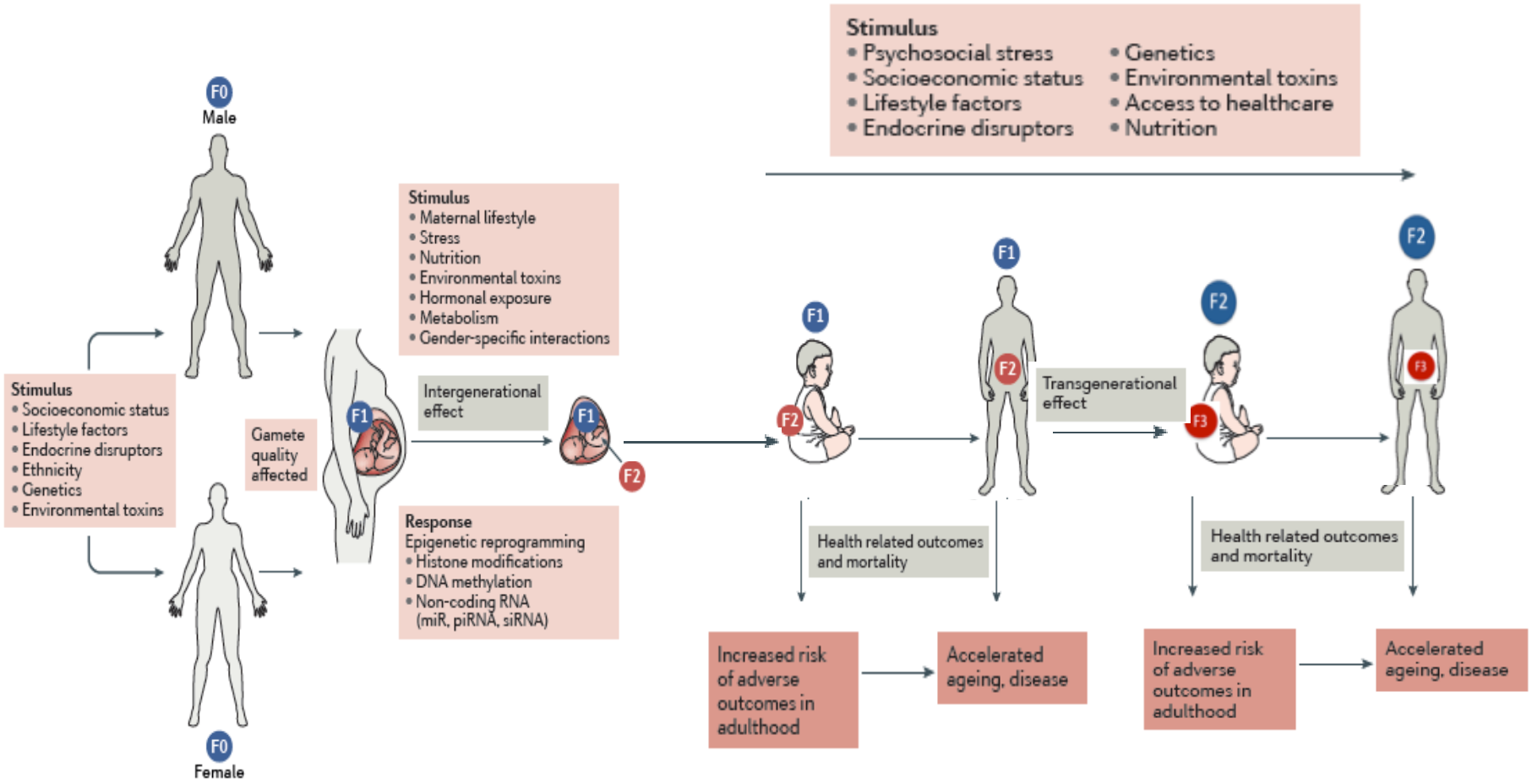
Figure 2. Environmental factors and epigenomic alterations.

Epigenomic changes enable heritable genomic plasticity to environmental stressors. The figure illustrates how these can be generated by exposure to a range of diverse stimuli, resulting in germline (trans-generational) or somatic cells (inter-generational) during pre-natal development, which subsequently influences adult health outcomes. Stimuli can also have gender specific effects. DDT for example affects fertility in both genders, but only in males is there a specific effect on the kidney, causing a change in kidney size in murine models. Multigenerational exposure to the initial stimulus and a subsequent intergenerational effect is not observed beyond the F2 generation. Trans-generational inheritance has a permanent phenotypic effect in F3 (females/F2 males) (unexposed) and beyond. This is not well documented in humans mostly due to the complex physiology and complexity of maternal effects during/after gestation (intergenerational effects).

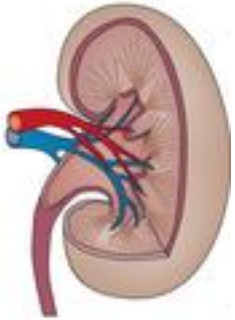
Figure 3. Aging and epigenomic changes in the kidney.

There is a renal phenotype common to aged and CKD kidneys[1], where epigenomic changes parallel aging and accelerated aging in CKD. These reflect epigenetic responses to exogenous and endogenous stimuli and nutrient availability through the life course concomitant with increasing allostatic load. These responses constitute changes in a dynamic series of interrelated regulator networks linking the chromatin dynamics with the methylome, microtranscriptome, transcriptome and proteome. This network is dysregulated and impaired by a range of stressors, including the SASP, and CPPs, resulting in further epigenetic changes, impaired immune and anti-aging defenses and metabolic dysfunction.

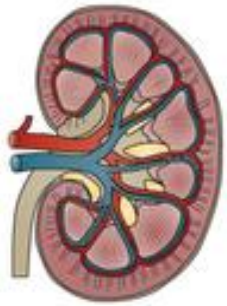

Risk factors for renal dysfunction
Exogenous origin
Socioeconomic
Psychological
Lifestyle factors
Access to healthcare
Environmental toxins
Endocrine disruptors
Hormonal exposure
Intrauterine environment
Pathogens
Endogenous origin
Uremic insults
(uremic toxins, chronic inflammation, dyslipidemia, oxidative stress, hyperphosphatemia, hyperhomocysteinemia)
Hormonal exposure
Gut dysbiosis
Gender/gender-specific factors
Genotype
Cellular senescence
Chronological aging
Increasing allostatic load over the life course
Features of Epigenetic landscape affecting renal function
Post-translational histone modifications
(acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, malonylation)
Chromatin remodelling
DNA methylation changes
RNA methylation changes
Non-coding RNA changes
(lncRNA, miRNA, piRNA, siRNA)
Functional consequences in cellular biology
Aberrant gene expression
Lost of imprinting
Chromosomal instability
Telomere attrition
Metabolic shift
Mitochondrial dysfunction
Autophagy
Proteostasis
Cellular senescence
SASP (Low grade chronic inflammation)
Nuclear envelope dysfunction
Circadian rhythm dysregulation
Low Klotho expression
Defective immune system
CPP induced cellular dysfunction
Phenotype and Dysfunction
Accelerated aging
Vascular disease
Insulin resistance
Reduced renal function
Chronic kidney disease
Frailty
Muscle wasting
Osteoporosis
Cognitive dysfunction
Disease outcome and progression


Stimulus
• Psychosocial stress
• Socioeconomic status
• Lifestyle factors
• Endocrine disruptors
• Genetics
• Environmental toxins
• Access to healthcare
• Nutrition
F0
Male
F0
Female
Stimulus
• Socioeconomic status
• Lifestyle factors
• Endocrine disruptors
• Ethnicity
• Genetics
• Environmental toxins
Gamete quality affected
F1
Stimulus
• Maternal lifestyle
• Stress
• Nutrition
• Environmental toxins
• Hormonal exposure
• Metabolism
• Gender-specific interactions
Intergenerational effect
F1
F2
Response
Epigenetic reprogramming
• Histone modifications
• DNA methylation
• Non-coding RNA (miR, piRNA, siRNA)
F1
F2
F1
F2
Transgenerational effect
F2
F3
F2
F3
Health related outcomes and mortality
Health related outcomes and mortality
Increased risk of adverse outcomes in adulthood
Accelerated ageing, disease
Increased risk of adverse outcomes in adulthood
Accelerated ageing, disease

Young kidney



Old kidney/ CKD



Exogenous factors/endogenous factors

ncRNA

Chromatin remodelling

Histone composition changes
Histone post-translational modification shift (H3 methylation pattern)
Nucleosome
Heterochromatin/Euchromatin status

DNA methylation

Transcription factors binding
Transcriptional co-factors availability

Transcriptome

Non somatic mutation
RNA methylation/transcript stability
Alternative splicing
Ribosomes

Proteome

Translation
Post-translational modifications

- Telomere attrition
- Deficient response to DNA damage
- Epigenetic changes
- Metabolic shift
- Mitochondrial dysfunction
- Defective ER stress responses
- Decline in autophagy
- Defective proteostasis
- Decline in regenerative capacities
- Stem cell exhaustion
- SASP (Low grade chronic inflammation)
- Cellular senescence
- Accumulation of damaged cells
- Damage to nuclear envelope (lamina)
- Decline in renal function
- Impaired immune defence
- Low Klotho expression
- Calciprotein particle (CPP) toxicity

Aging trajectory with associated increase in allostatic load