Mitochondria and Chronic Kidney Disease: A Molecular Update

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Abstract

Chronic kidney disease (CKD) has become a worldwide public health priority and is estimated to affect approximately 12% of the global population. CKD is associated with an increased cardiovascular morbidity, premature mortality, and a substantial economic burden.

Increased generation of reactive oxygen species has been observed throughout CKD progression, suggesting that mitochondrial dysfunction may be important in the pathogenesis of kidney disease. The mitochondrial genome is a circular double stranded DNA molecule composed of 16,569 base pairs harbouring 37 genes, which encode 13 key proteins of the electron transport chain along with two rRNAs and 22 tRNAs. At least 2,309 nuclear genes are also necessary for efficient mitochondrial function. Mitochondrial dysfunction leads to a reduction in ATP production, cellular damage and loss of renal function. Damage or mutations in mtDNA will lead to defects in mitochondrial oxidative phosphorylation (OXPHOS), which can result in a range of clinical symptoms involving several different organs broadly termed as ‘mitochondrial diseases’. Defects in nuclear encoded genes may also lead to OXPHOS defects, abnormal protein translation and loss of mtDNA copy number.

This review provides an update on molecular features influencing mitochondrial homeostasis and function, highlighting how these are compromised during CKD.
Introduction

Chronic kidney disease (CKD) is a worldwide public health priority and is estimated to currently affect between 11–13% of the global population.\(^1\) CKD is associated with increased cardiovascular morbidity, premature mortality and a substantial healthcare cost.\(^2,3\) Gradual decline in kidney function in individuals with CKD involves dysfunction of several biological pathways, including altered cellular metabolism, nitrogen balance and protein metabolism changes, insulin resistance and increased production of mediators of inflammation and oxidative stress.\(^4-7\) Increased generation of reactive oxygen species (ROS) has been observed at throughout progressive stages of CKD and it has been suggested that this may be the result of mitochondrial dysfunction.\(^8\)

Mitochondria are responsible for generation of adenosine triphosphate (ATP) to provide energy to eukaryotic cells \textit{via} a series of oxidative phosphorylation (OXPHOS) reactions known as the electron transport chain (ETC). Mitochondria also regulate cellular metabolism \textit{via} heme and steroid synthesis, generating ROS, establishing the membrane potential and controlling calcium and apoptotic signalling.\(^9,10\) It is thought that these cellular powerhouses (Fig. 1) originated through an endosymbiotic relationship with an \(\alpha\)-proteobacterium.\(^11\) Although many mitochondrial genes are now located on nuclear chromosomes these organelles contain their own circular genome reminiscent of their bacterial ancestors.\(^12,13\) By transferring a substantial amount of genetic material to the nuclear genome the size of the mitochondrial genome is greatly reduced, which may impart a replicative advantage and reduce the likelihood of incurring disadvantageous mutations, thus reinforcing the idea of a synergistic relationship between these two genomes.\(^14\)

Among human body organs, the kidneys are second to the heart in terms of energy demands, mitochondrial content and oxygen consumption.\(^15,16\) This is necessary to produce the energy required for removal of waste from blood, reabsorption of nutrients, regulation of electrolyte and fluid balance, maintenance of acid–base homeostasis, and regulation of blood pressure.\(^17\) Mitochondria also provide the energy required by \(\text{Na}^+-\text{K}^+\text{-ATPase}\) to create ion gradients across the cellular membrane and to facilitate active transport in the proximal tubule, the loop of Henle, the distal tubule and the collecting duct to allow ion reabsorption and excretion.\(^18\) Energy demands, and in turn mitochondrial content are much higher in the proximal tubules compared with the glomerulus as glomerular filtration is a passive process whereas the proximal tubules require a large variety of active transport mechanisms in order to reabsorb 80% of the filtrate that passes through the glomerulus.\(^19\) Because of these high energy requirements, mitochondrial dysfunction in the kidneys may severely impact renal health and has previously been implicated in CKD development.

CKD is typically defined as kidney structure or function abnormalities persisting for more than three months and impacting on the health of the individual.\(^20\) As the body ages the kidneys undergo age-related structural changes along with a reduction in functional capacity. In adults over 35 years, kidneys gradually lose functional nephrons and decrease in size so that by 80 to 85 years old many individuals will have lost up to 30% of total kidney mass.\(^21\) Although many older people with reduced kidney mass will continue to have normal kidney function there is a reduction in the “margin of safety” which in turn will impact on the kidneys’ ability to respond to stress placed upon remaining nephrons such as infection or reduced blood flow.\(^21\) Many elderly people will exhibit a low
glomerular filtration rate (GFR) but without any specific kidney disease. It is therefore important to understand the underlying genetic causes of kidney disease and decreasing renal function in order to design more effective therapies and reduce the associated healthcare burden. Persistent reduced kidney function, with or without proteinuria measured by albumin creatinine ratio (ACR), is indicative of CKD and is associated with damage to kidney tubules and glomeruli. Although the underlying cause of reduced renal function in CKD may vary between individuals, there are several pathways involved with CKD development including accumulation of extracellular matrix (ECM) proteins in the glomerulus, interstitial fibrosis, tubular atrophy and inflammation. Over the last decade, several genetic risk factors have been identified and robustly associated with CKD.

**Mitochondrial Genetics**

Genes required for normal mitochondrial function are found on both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). The mitochondrial genome is a circular double stranded DNA molecule composed of 16,569 base pairs harbouring 37 genes encoding 13 key proteins of the ETC along with two rRNAs and 22 tRNAs (Fig. 1). In contrast to nDNA, mtDNA lacks introns and non-coding intergenic regions, apart from a small regulatory region between the mitochondrial genes for phenylalanine and proline tRNAs known as the non-coding region (NCR). This 1.1 kb stretch of DNA frequently contains a third single strand of DNA 650 nucleotides long (7S DNA) which forms a displacement-loop, or D-loop, postulated to be an mtDNA replication intermediate, facilitating more open conformation of the mtDNA molecule, allowing proteins involved in replication or transcription to bind and regulate these activities. Although the exact mechanism of replication in mtDNA is not yet fully understood, a number of nuclear encoded enzymes are essential to this process.

In humans, mtDNA passes to offspring through maternal gametes. As each mitochondrion contains multiple copies of maternally inherited mtDNA, and each cell contains many mitochondria, it is possible for mutations to occur during replication which will affect some mtDNA molecules but not others; this phenomenon is known as heteroplasmy and contributes to disease development. During an individual’s lifetime the actions of ROS and the resulting mtDNA mutations are thought to be directly involved in various disease mechanisms and the process of aging.

There are at least 2,526 autosomal genes involved with mitochondrial function. Many nuclear encoded mitochondrial genes (NEMGs) code for proteins which are synthesised on ribosomes in the cytosol, migrate to the mitochondria, and are transported across the mitochondrial membrane based on the presence of a specific N-terminal presequence. Other NEMGs do not code directly for mitochondrial components but regulate the expression of a range of genes which are essential for normal mitochondrial biogenesis and function.
Mitochondria and nuclear gene interactions

Mitochondrial dysfunction may be due to inherited germline or acquired somatic mutations. For example, acquired damage to tubular mitochondria alters mitochondrial dynamics, mitophagy and biogenesis in acute kidney injury which is risk factor subsequently for CKD. RecQ-like helicase 4 (RECQL4) is a dynamic protein important for protein-protein interactions in nDNA replication but also localises to the mitochondrion where it interacts with POLγ in order to maintain mtDNA integrity. RECQL4 mutations result in increased mtDNA copy number and mitochondrial dysfunction. This protein may also act to regulate p53 tumour mitochondrial transport while RECQL4 dysfunction may decrease p53 activity. Data from immunofluorescence microscopy suggest that DNA2 molecules are mainly found in mitochondria where they have been shown to interact with Poly and Twinkle. These interactions may be important in repairing oxidative lesions in mtDNA, mtDNA maintenance and protection against mtDNA related diseases. Petite integration frequency 1 (PIF1) is a member of the superfamily 1 helicase family that acts in both nuclei and mitochondria. A DExH-box helicase known as suppressor of Var1 3-Like Protein 1 (SUV3) is thought to be indirectly involved in mtDNA stability and copy number regulation. SUV3 is important in regulating mitochondrial RNA levels and may also interact with nDNA replication and repair factors in the nucleus.

These interactions between mtDNA and nDNA highlight the symbiotic relationship between mitochondrial organelle and the nucleus. Due to this relationship, dysfunction of genes in mtDNA or nDNA can have devastating consequences and mitochondrial dysfunction is strongly implicated in kidney disease.

Mitochondria and CKD

More than 250 genes involved with mitochondrial energy metabolism have been associated with human disease and this mitochondrial damage may occur due to direct mtDNA insult or NEMG defects. Damage or mutations in mtDNA may lead to primary defects in mitochondrial OXPHOS due to dysfunction of the ETC components, which can result in a range of clinical symptoms involving several different organs broadly termed as, ‘mitochondrial diseases’. Mutations in mtDNA often have a greater functional impact than variants in nuclear-encoded mitochondrial genes; several mtDNA genes are known to affect kidney function (Table 1).

For example, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome are mitochondrial cytopathies resulting from defects in MT-ND1 and MT-ND5 which encode for complex I proteins, as well as MT-TH, MT-TL1, and MT-TV which code for mitochondrial tRNAs. NEMG insults may also lead to OXPHOS defects, abnormal protein translation, and lower mtDNA copy number. Traditional mitochondrial diseases often include renal complications, due to an mitochondrial abundance as well as kidney high energy demands e.g. defects in CoQ₁₀ synthesis and the mtDNA 3243 A>G mutation are known to cause renal complications including focal segmental glomerulosclerosis. Tubular defects are the most common renal manifestation of
mitochondrial disorders from mutations in mtDNA or NEMGs. Mitochondrial isoleucine tRNA gene (tRNA\textsubscript{Ile}) mutations have been associated with familial hypercholesterolemia and hypomagnesaemia.\textsuperscript{73} Fanconi syndrome which is secondary to kidney tubular dysfunction has been linked with mutations in the gene \textit{EHHADH}.\textsuperscript{74} Five nuclear genes (\textit{COQ5, COX6A1, GATC, TOP1MT} and \textit{PARCRG}) have been linked to kidney disease in people with type 1 diabetes, across multiple cohorts.\textsuperscript{75}

Thirty-eight NEMGs are reported to be involved with CKD development (Table 2; Supplementary Table 1), with many having important roles in maintaining mitochondrial function and renal health. NEMGs dysregulation because of acquired or inherited mutations can affect several pathways which may initiate a vicious cycle aggravating renal damage and leading to CKD (Fig. 2). Many NEMGs are essential for mitochondrial biogenesis and normal mitochondrial function, particularly \textit{PPARGC1A} which regulates \textit{TFAM, COX6C, COX7C, UQCRH, MCAD, SIRT3}, and \textit{NRF1}.\textsuperscript{76} \textit{PPARGC1A} and its downstream targets are reported to be downregulated in peripheral blood mononuclear cells of CKD patients. \textit{PPARGC1A} induction by ROS may act as a protective adaption to reduce further ROS generation.\textsuperscript{77}

Other genes involved with mitochondrial biogenesis are upregulated in CKD in response to increased ROS production, these include \textit{NFE2L2} (regulated by \textit{PPARGC1A}), \textit{SOD2}, Complex 1 components (\textit{NDUFS5, NDUFA6, NDUFA1} and \textit{NDUFB1}), \textit{UQCRH, UQCRB, ATPSI, ATPSJ}, and \textit{ATPSO}.\textsuperscript{77–79} Upregulation of these genes may be an attempt to compensate for increased ROS generation resulting from OXPHOS dysfunction. Genes coding for complex IV 6C and 7C subunits (\textit{COX6C} and \textit{COX7C}) were also seen to be upregulated in CKD, however, complex IV activity was reduced due to chronic oxidative stress and oxidant injury resulting from OXPHOS system inhibition.\textsuperscript{11} Damage to nDNA and mtDNA genes may have an impact on mitochondrial function due to ineffectual clearance of ROS which may further exacerbate mitochondrial damage and lead to increased ROS production.

Several studies have investigated SNPs associated with chronic kidney disease (Table 3). Damage to both NEMGs and mtDNA genes may have an impact on mitochondrial function due to ineffectual clearance of ROS which may further exacerbate mitochondrial damage and lead to increased ROS production due to \textit{AIFM1}\textsuperscript{80} downregulation and \textit{NOX4} upregulation.\textsuperscript{81,82} This vicious cycle of ROS production results in oxidative stress within the mitochondria leading to \textit{ATG5} and \textit{BECN1} downregulation,\textsuperscript{83} which may lead to abnormal or impaired autophagy which in turn leads to further ROS generation, dysregulated mitochondrial fission and reduced mitochondrial function.\textsuperscript{83–85} \textit{BNIP3} is an important mediator of mitophagy and upregulation of this gene has been associated with sarcopenia in CKD along with decreased mtDNA copy number and reduced mitochondrial function.\textsuperscript{86} The intrinsic pathway of apoptosis has also been implicated in CKD. Downregulation of the anti-apoptotic protein \textit{BCL-xL} coded for by \textit{BCL2L1} combined with upregulation of pro-apoptotic \textit{BAX} and \textit{BAK1} leads to increased apoptosis and depolarisation of the outer mitochondrial membrane in proximal tubular cells. \textit{BAX} upregulation and \textit{BCL2L1} downregulation activates the intrinsic apoptotic pathway, permeabilising the inner mitochondrial membrane leading to cytochrome C release.\textsuperscript{87–89}
Uncontrolled apoptosis may eventually lead to inflammation and fibrosis commonly observed in CKD. Experimental CKD models have shown increased expression of several genes involved with inflammation and fibrosis. TGFβ expression increases in response to apoptosis in primary tubular cells exposed to albumin stimulating ECM formation and the extrinsic apoptotic pathway.\(^{90,91}\) \(PPARγ\) upregulation is common in several forms of renal disease and affects renal parenchymal cells stimulating various pathways important in CKD development including fibrotic, inflammatory, immune, proliferative, reactive oxygen and mitochondrial injury pathways.\(^{92}\) \(NLRP3\) upregulation and downstream targets \(CASP1\), IL-18, and IL-1β are upregulated in CKD in response to mitochondrial ROS generation. Activation of the NLRP3 inflammasome leads to increased ECM deposition, apoptosis and fibrosis of renal cells. \(NLRP3\) knockout or ROS inhibition reduce mitochondrial dysfunction, reduce apoptosis, decrease ECM deposition and protect against renal fibrosis.\(^{93–96}\)

Mitochondrial dysfunction resulting from inherited or acquired genetic defects may lead to development of various forms of kidney disease such as acute kidney injury, diabetic nephropathy, glomerular diseases, tubular diseases and CKD.\(^{43,97,98}\) In summary, mitochondrial dysfunction andOXPHOS defects may increase ROS generation and reduce ATP production which leads to increased oxidative stress which may lead to uncontrolled autophagy, mitophagy and further ROS production. Mitochondrial dysfunction, ROS generation and the resulting dysregulation of autophagic mechanisms may also lead to an upregulation of the intrinsic pathway of apoptosis which in turn leads to inflammation and fibrosis in the renal tubules, glomerulus and podocytes. This damage may then generate further autophagy leading to apoptosis, fibrosis and inflammation with further podocyte function reduction eventually leading to irreversible podocyte injury and progression to CKD.

NEMGs involved with podocyte function may play a key role in the progression of CKD. Cathepsin D, coded for by \(CTSD\), is a lysosomal proteinase involved with lysosomal degradation, autophagic degradation and contributes to maintaining podocyte homeostasis. \(CTSD\) may be downregulated or inactivated in CKD and loss of this protein leads to impaired autophagy, resulting in the accumulation of toxic subunit c–positive lipofuscins and slit diaphragm proteins followed by apoptotic cell death.\(^{99}\) \(ITCH\) expression is increased in mouse models of kidney disease.\(^{100}\) This gene is regulated by the Src kinase Fyn and modulates various signalling pathways including TGFβ and EGF through ubiquitin and non-ubiquitin mediated mechanisms.\(^{101–104}\) Interactions with Fyn, TGFβ and related signalling molecules may suggest a role of \(ITCH\) in regulating glomerular sclerosis and podocyte function.\(^{100}\) Downregulation of \(NRP2\) was observed in animal models of kidney disease and NRP2 knockout mice displayed progressive glomerular damage when exposed to a podocyte toxin.\(^{100,105}\)

**Epigenetic features affecting mitochondrial in CKD**

Epigenetic modifications affect gene expression without directly altering the DNA sequence. These changes can be inherited between generations or may result from environmental exposures acquired during a lifetime.\(^{106,107}\) Known epigenetic mechanisms include DNA methylation,\(^{108,109,110}\) histone modifications,\(^{111}\) regulation by non-coding RNA, and chromatin remodelling.\(^{112–114}\) Epigenetic
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modifications resulting from an adverse in utero environment have been implicated in increased risk of several diseases such as cardiovascular disease, hypertension, type 2 diabetes mellitus, obesity and renal disease.\textsuperscript{115–120} A systematic review by White and colleagues\textsuperscript{121} reported that low birth weight individuals (<2.5 Kg at birth) have an increased CKD risk of in adulthood. Epigenetic changes are also acquired throughout life often in response to adverse conditions such as the high-glucose environment experienced in diabetes mellitus.\textsuperscript{122} The role of epigenetic modifications in kidney disease have previously been discussed in detail elsewhere\textsuperscript{123–126} and methylation of nuclear DNA and histone modifications are known to influence CKD development, particularly in patients with diabetes.\textsuperscript{127,128} Genes involved with renal development and renal fibrosis have also been found to be differentially methylated in persons with CKD.\textsuperscript{129–135} Amongst these differentially methylated genes the top canonical pathways included oxidative phosphorylation and mitochondrial dysfunction.\textsuperscript{135} DNA hypermethylation was also observed on the PGC-1α promotor region in diabetic patients and was inversely correlated with mitochondrial content.\textsuperscript{136,137} Recent studies using cell lines devoid of mitochondria demonstrated that depletion of mitochondrial DNA can lead to abnormal CpG methylation patterns and restoration of mtDNA in these cells partially reverse these changes.\textsuperscript{138} This highlights the ability of mitochondria and nDNA to interact through both genetic and epigenetic mechanisms. Due to this complex interaction, mitochondrial dysfunction will affect both the nuclear and the epi-genome. Resulting bioenergetics failure is implicated in a number of energy deficiency diseases particularly in tissues which rely on high energy flux including brain, heart, muscle, renal, and endocrine systems.\textsuperscript{139,140} Within the context of renal disease, mitochondrial proteins ability to cause epigenetic modifications has been demonstrated through the ability of mitofusion 2 to abate histone acetylation in the promoter region of collagen IV through a reduction in ROS generation, in turn reducing collagen IV expression in streptozotocin-induced diabetic rats.\textsuperscript{141–144} 

Historically, there was much debate surrounding the mtDNA ability to undergo epigenetic changes.\textsuperscript{145–151} However, in 2011 advances in methodology and sensitivity demonstrated the presence of methylated bases in human mtDNA.\textsuperscript{152} Shock and colleagues showed that a DNMT1 transcript variant was present inside mitochondria where it is capable of modifying transcription of the mitochondrial genome.\textsuperscript{153} Also around this time, Chestnut and colleagues observed DNMT3a in mitochondria of mouse and human tissue, providing evidence that DNA methylation may act on mtDNA by similar mechanisms as in nDNA.\textsuperscript{154} In mtDNA methylated cytosines are mainly located in non-CpG moieties within the mitochondrial D-Loop, particularly in the promotor region of the heavy strand and in conserved sequence blocks of the which may indicate a role in regulating mtDNA replication or transcription.\textsuperscript{155} In addition to cytosine methylation mtDNA also contains hydroxymethylated cytosine at a higher density than in autosomes in both the D-Loop region and along the entire mitochondrial genome.\textsuperscript{155,156} MtDNA is protein coated and contained within nucleoids.\textsuperscript{157–159} Histone family members, particularly H2A and H2B, have also been observed in mitochondria although rather than directly binding DNA these were found to localise to the mitochondrial membrane.\textsuperscript{160} In 2008 Bogenhagen and colleagues\textsuperscript{161} found 57 proteins associated with human mtDNA. Amongst these, there are several proteins essential for mitochondrial biogenesis as well as for mtDNA transcription and replication including Mitochondrial transcription factor A, mitochondrial transcription factor B, DNA polymerase gamma, prohibitin, Twinkle helicase and mitochondrial single-stranded DNA binding protein.\textsuperscript{159,162–165} Whole genome and transcriptome sequencing has also revealed the presence of numerous ncRNAs including siRNAs, miRNAs and lncRNAs which are involved with regulating essential signalling pathways in mitochondria by altering expression of nuclear-encoded mitochondrial proteins.\textsuperscript{166–172} In addition to ncRNAs encoded by
nDNA, Rackham and colleagues\textsuperscript{173} identified three lncRNAs transcribed by the mitochondrial genome from regions complementary to ND5, ND6 and Cytb genes. These mitochondrial lncRNAs are regulated by the mitochondrial RNase P complex and their abundance varies significantly across different tissue types with a high abundance observed in cardiac tissue.\textsuperscript{173,174} There are also thousands of non-coding small RNAs transcribed by mtDNA the majority of which are derived from sense transcripts of mitochondrial genes, but these have also been mapped to the mitochondrial D-loop region.\textsuperscript{175,176}

In addition to external environmental stress, altered metabolic states such as hyperglycaemia in diabetes or uraemia in CKD can also lead to epigenetic changes known as hyperglycaemic or uremic “memory”, respectively.\textsuperscript{177–179} Epigenetic features are potential therapeutic targets as they may be reversible and tissue specific, therefore they may be attractive potential foci for drugs targeting specific epigenetic changes. For example, epigenetic changes associated with diabetic nephropathy can be reversed by losartan treatment in diabetes mouse models.\textsuperscript{123,180–182}

**Conclusions**

Persistent mitochondrial dysfunction is known to be involved in the initiation and progression of renal diseases, such as acute kidney injury and diabetic nephropathy, resulting from disruption to mitochondrial homeostasis and normal kidney function.\textsuperscript{19} Oxidative stress is a common CKD feature and increased production of reactive oxygen species due to mitochondrial dysfunction has been observed in diabetes, inflammation and aging.\textsuperscript{183} Increased ROS production due to mitochondrial dysfunction may also contribute to CVD and other co-morbidities associated with CKD.\textsuperscript{86,183,184} Further investigation into mitochondria roles in maintaining renal health and their contribution to various forms of CKD may unveil new disease mechanisms as well as novel treatments. Significant genetic damage or mutations in NEMGs may also disrupt mitochondrial homeostasis. The cellular machinery and related genes involved in mitophagy, mitochondrial fission, fusion and biogenesis are essential in maintaining mitochondrial homeostasis and dysfunction of these processes may lead to irreversible kidney damage. There is evidence to suggest that mitochondrial dysfunction precedes CKD development, occurring in the early stages of acute kidney injury and diabetic nephropathy, failure to restore mitochondrial function may then initiate the vicious cycles outlined earlier resulting in CKD.\textsuperscript{185} The use of multi-omic approaches to investigate CKD are providing valuable insights; the integrated use of these techniques will allow a better understanding of mitochondria influencing in renal disease.

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region of genes encoding p22phox (CYBA) and glutamate cysteine ligase catalytic subunit (GCLC) and renal disease in patients with type 1 diabetes mellitus. *BMC Med Genet.* 2011;12:129.


Table 1: Defects in Mitochondrial DNA previously associated with kidney complications

<table>
<thead>
<tr>
<th>Variation Type</th>
<th>Variation</th>
<th>Region</th>
<th>Phenotype</th>
<th>References</th>
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<tbody>
<tr>
<td>Point mutation</td>
<td>mtDNA 3243 A&gt;G mutation</td>
<td>MT-TL1</td>
<td>MELAS, MERRF syndrome and MIDD</td>
<td>67,78,186–188</td>
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<td>Point mutation</td>
<td>c.550A&gt;G</td>
<td>MRPS7</td>
<td>Substitution of valine for a highly conserved methionine (p.Met184Val) observed in case report of Congenital sensorineural deafness, progressive hepatic and renal failure and lactic academia</td>
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<tr>
<td>Point mutation</td>
<td>m.12425delA</td>
<td>MTND5</td>
<td>Renal failure and myopathy resulting from Complex I deficiency</td>
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<td>Point mutation</td>
<td>m.G586A</td>
<td>mt-tRNA&lt;sup&gt;Phe&lt;/sup&gt;</td>
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<td>m.A608G</td>
<td>mt-tRNA&lt;sup&gt;Phe&lt;/sup&gt;</td>
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<td></td>
<td>m.3243</td>
<td>mt-tRNA&lt;sup&gt;Leu&lt;/sup&gt;</td>
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<td></td>
<td>m.A5666G</td>
<td>Noncoding</td>
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<td>Point mutation</td>
<td>m.G3242A</td>
<td>mt-tRNA&lt;sup&gt;Leu&lt;/sup&gt;</td>
<td>Renal failure, RTA type 4</td>
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<td>Point mutation</td>
<td>m.3243</td>
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<td>FSGS</td>
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<td>m.A4269G</td>
<td>mt-tRNA&lt;sup&gt;Met&lt;/sup&gt;</td>
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<td>mt-tRNA&lt;sup&gt;Asn&lt;/sup&gt;</td>
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<td>m.A5843G</td>
<td>mt-tRNA&lt;sup&gt;Tyr&lt;/sup&gt;</td>
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<td>Point mutation</td>
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<td>ND5</td>
<td>Glomerulocystic disease, renal failure</td>
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<td>Deletion</td>
<td>6,000 bp</td>
<td>~6,000–12,000</td>
<td>Proximal tubulopathy</td>
<td>206</td>
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<tr>
<td>Deletion</td>
<td>7,500 bp</td>
<td>~6,100–13,600</td>
<td>FSGS, RTA</td>
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<td>Deletion</td>
<td>8,800 bp</td>
<td>~6,800–15,600</td>
<td>Distal tubulopathy</td>
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<td>Deletion</td>
<td>7,315 bp</td>
<td>7,325–14,639</td>
<td>Proximal tubulopathy, TIN</td>
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<td>2,800 bp</td>
<td>~10,000–12,800</td>
<td>Proximal tubulopathy, TIN</td>
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<td>5,700 bp</td>
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<td>4,977 bp</td>
<td>8,469–13,447</td>
<td>Proximal tubulopathy</td>
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<td>2,608 bp</td>
<td>10,598–13,206</td>
<td>TIN</td>
<td>214</td>
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<td>SNPs</td>
<td>73A&gt;G</td>
<td>mtDNA D-loop 1122 bps from nucleotide 16,024–16,569 and 1–576</td>
<td>These D-loop SNPs are associated with risk of CKD and kidney survival time in those with CKD. SNPs in this region may affect mtDNA replication and lead to electron transport chain alteration, therefore increasing ROS generation and may contribute to nuclear genome damage.</td>
<td>215</td>
</tr>
</tbody>
</table>

MELAS - Mitochondrial Encephalopathy, Lactic acidosis, and Stroke-like episodes; MERRF syndrome - Myoclonic epilepsy with ragged-red fibers; MIDD - Maternally-inherited diabetes and deafness; TIN – Tubulointerstitial nephritis; RTA – Renal Tubular Acidosis; FSGS - Focal segmental glomerulosclerosis; SNP – Single Nucleotide Polymorphism; CKD – Chronic Kidney Disease; ROS – Reactive Oxygen Species
Table 2 Nuclear encoded mitochondrial genes involved with development of chronic kidney disease (more details in supplementary table 1)

<table>
<thead>
<tr>
<th>Biological Process</th>
<th>Genes and expression in CKD</th>
<th>Pathological Effect of gene expression</th>
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<tbody>
<tr>
<td>Mitochondrial Biogenesis and Function</td>
<td>Downregulated: PPARGC1A, NRF1; TFAM; UQCRH; COX6C and COX7C in PBMC of CKD patients undergoing PD; MCAD; AIFM1</td>
<td>PPARGC1A downregulation may be protective adaption to limit ROS production. Will also reduce expression of downstream targets of NRF-1 and PGC1α and reduce mitochondrial biogenesis and OXPHOS activity. Downregulation of TFAM may lead to decreased mtDNA copy number. Expression of NFE2L2, SOD2 and UQCRH may also be associated with increased ROS generation. Increased expression of Complex I proteins. Complex V proteins and UQCRB may be an attempt to restore OXPHOS mechanisms. Reduced Complex IV activity may be associated with OXPHOS defects. Increased expression of renal risk variants of APOL1 and NOX4 may increase mitochondrial damage by ROS.</td>
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<td>Upregulated: COX6C and COX7C in PBMC of CKD (stage IV – V) patients in conservative treatment and HD; NFE2L2; SOD2; NDUFS5; NDUFA6; NDUFA1; NDUFB1; UQCRB; ATP5I; ATP5J; ATP5O; APOL1; NOX4</td>
<td></td>
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<tr>
<td>Apoptosis (Intrinsic Pathway)</td>
<td>Downregulated: BCL2L1; AIFM1</td>
<td>Reduced expression of BCL-xL allows BAX expression and mitochondrial accumulation to increase which increases apoptosis and depolarises outer mitochondrial membrane in proximal tubular cells. Increased BAX expression also permeabilises the inner mitochondrial membrane leading to cytochrome C release. AIFM1 inactivation results in OXPHOS defects. Activation of HIF1 promotes renal cell growth, stimulates angiogenesis, and reduces inflammation.</td>
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<tr>
<td></td>
<td>Upregulated: BAX; BAK1; HIF1</td>
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<tr>
<td>Autophagy and Mitophagy</td>
<td>Downregulated: ATG5; CTSD; BECN1</td>
<td>Downregulation of ATG5; CTSD; BECN1 may lead to impaired autophagy and increased ROS production, mitochondrial deformation and decreased mitochondrial function. Increased expression of BNIP3 may indicate increased mitophagy and reduced mitochondrial function.</td>
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<tr>
<td></td>
<td>Upregulated: BNIP3</td>
<td></td>
</tr>
<tr>
<td>Inflammation and fibrosis</td>
<td>Upregulated: NLRP3; CASP1; PPARG; TGFβ</td>
<td>NLRP3 inflammasome is activated by mitochondrial ROS which, increases ECM deposition, apoptosis, fibrosis of renal cells and CASP1 expression. Uprgulation of PPARG effects renal parenchymal cells a well as fibrotic, inflammatory, immune, proliferative, reactive oxygen and mitochondrial injury pathways. Apoptosis in Primary tubular cells exposed to albumin has been shown to increase TGFβ expression which stimulates ECM formation and the extrinsic apoptotic pathway.</td>
</tr>
<tr>
<td>Podocyte Function</td>
<td>Downregulated: NRP2; KLF6; CTSD</td>
<td>Downregulation of NRP2 in CKD may contribute to podocyte damage. Podocyte specific KLF6 loss increased susceptibility to FSGS in mice models. Downregulation of CTSD in podocytes may lead to impaired autophagy and eventually apoptotic cell death.ITCH expression may be important in regulating glomerular sclerosis and podocyte function.</td>
</tr>
<tr>
<td></td>
<td>Upregulated: ITCH</td>
<td></td>
</tr>
</tbody>
</table>

PBMC – Peripheral blood mononuclear cell; CKD – Chronic kidney disease; PD – peritoneal dialysis; HD - haemodialysis; ROS – Reactive oxygen species; OXPHOS – Oxidative phosphorylation; mtDNA – mitochondrial DNA; ECM – Extracellular matrix
Table 3 Examples of SNPs in nuclear encoded mitochondrial genes previously associated with chronic kidney disease

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene</th>
<th>Top Ranked SNPs</th>
<th>Statistics</th>
<th>Protein function in Mitochondria</th>
<th>Population</th>
<th>Notes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR</td>
<td>NAT8</td>
<td>rs13538</td>
<td>P = 4.5 x10^{-14}</td>
<td>May be involved in regulation of apoptosis</td>
<td>Meta Analyses of GWAS from individuals of European ancestry</td>
<td>Common variants in NAT8 may influence acetylation pathways, disturbances of which are known to be involved with drug and toxin induced kidney injury.</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>SLC22A2</td>
<td>rs2279463</td>
<td>P = 5.5 x10^{-12}</td>
<td>Evidences for mitochondrial localisation</td>
<td>Mediates cation reabsorption in kidney</td>
<td>Mediates cation reabsorption in kidney</td>
<td>216</td>
</tr>
<tr>
<td></td>
<td>WDR72</td>
<td>rs139926232</td>
<td>P = 7.20 x10^{-11}</td>
<td>May be involved in localization of the calcium transporter SLC24A4 to the ameloblast cell membrane. Expressed in cytosol and mitochondria</td>
<td>Also involved with positive regulation of lipid metabolic process and abnormal glucose homeostasis</td>
<td>Also involved with positive regulation of lipid metabolic process and abnormal glucose homeostasis</td>
<td>216,217</td>
</tr>
<tr>
<td>Diabetic Nephropathy</td>
<td>AFF3</td>
<td>rs7583877</td>
<td>P = 1.2 x10^{-8}</td>
<td>Encodes a transcriptional activator, with DNA-binding activity</td>
<td>3 discovery cohorts from GENIE consortium: UK-ROI, FinnDiane and GoKinD US</td>
<td>Influences renal tubule fibrosis</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>MYO16-</td>
<td>rs9521445</td>
<td>P = 4.4 x10^{-3}</td>
<td>IRS2 has been seen to localise to mitochondria and is associated with DM</td>
<td>90% Caucasian from Joslin Clinic including patients from all social strata</td>
<td>These SNPs are found in an intergenic region telomeric to MYO16 and centromeric to IRS2. Associated with CKD in T2DM subjects. Not significant after Bonferroni correction</td>
<td>218</td>
</tr>
<tr>
<td>IRS2</td>
<td></td>
<td>rs1411766</td>
<td>P = 0.03</td>
<td>OR = 1.19 (95% CI: 1.01 - 1.40)</td>
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<td></td>
<td>COQ5</td>
<td>rs1167726</td>
<td>P = 2.00 x10^{-3}</td>
<td>Encodes methyltransferase which is located in mitochondrial matrix</td>
<td>All people included in the analysis were of white European origin and were diagnosed</td>
<td>Associated with both DKD and ESRD in the discovery and in silico replication analyses subjects with T1DM</td>
<td>25</td>
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<tr>
<td></td>
<td></td>
<td>rs614226</td>
<td>P = 2.00 x10^{-5}</td>
<td>Subunit 6A1 of cytochrome c oxidase which is the terminal</td>
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<tr>
<td></td>
<td>COX6A1</td>
<td>rs12310837</td>
<td>P = 3.00 x10^{-5}</td>
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<tr>
<td>Gene</td>
<td>SNP</td>
<td>P-Value</td>
<td>Description</td>
<td>Risk Factors</td>
<td>Reference</td>
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<tr>
<td>GATC</td>
<td>rs2235222</td>
<td>$P = 4.00 \times 10^{-5}$</td>
<td>Glutamyl-TRNA Amidotransferase Subunit C is required by mitochondria for formation of correctly charged Gln-tRNA(Gln)</td>
<td>with T1DM before the age of 31 years</td>
<td>[5]</td>
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<tr>
<td></td>
<td>rs7137953</td>
<td>$P = 4.00 \times 10^{-5}$</td>
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<tr>
<td>TOP1MT</td>
<td>rs724037</td>
<td>$P = 2.00 \times 10^{-5}$</td>
<td>Encodes a mitochondrial DNA topoisomerase that plays a role in the modification of DNA topology</td>
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<td>[5]</td>
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<tr>
<td>PACRG</td>
<td>rs2147653</td>
<td>$P = 0.004$</td>
<td>Coregulated with PARK2 which plays a key role in regulation of mitochondria function via mitophagy</td>
<td></td>
<td>[75]</td>
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<tr>
<td>CYBA</td>
<td>-675 T → A</td>
<td>$P = 0.0354$</td>
<td>CYBA encodes p22phox subunit which are essential for the function of NOX/NADPH oxidases</td>
<td>Age and gender matched Caucasian subjects with and without type 1 diabetes</td>
<td>[75]</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>OR = 0.38</td>
<td>C242T</td>
<td>CYBA -675 T → A modulate the risk for renal disease in the studied population of type 1 diabetes patients</td>
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<tr>
<td></td>
<td>C242T</td>
<td>$P = 0.015$</td>
<td>HR = 2.1</td>
<td>C242T correlates with microalbuminuria onset in the French T1DM cohort</td>
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<tr>
<td></td>
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<td></td>
<td>Modulates the risk for renal disease in the studied population of T1DM patients</td>
<td>Associated with cellular redox imbalances</td>
<td>[219]</td>
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<tr>
<td>GCLC</td>
<td>rs17883901</td>
<td>$P = 0.0068$</td>
<td>Modulates the risk for renal disease in the studied population of T1DM patients</td>
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<tr>
<td></td>
<td></td>
<td>OR = 2.40 CI 95%: 1.27-4.56</td>
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<tr>
<td>SIRT1</td>
<td>rs4746720 T&gt;C</td>
<td>$P = 0.041$</td>
<td>Involved with regulation of aging, longevity, and pathogenesis of age-related metabolic diseases, such as T2DM</td>
<td>Japanese cohort of T2Dm</td>
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<tr>
<td></td>
<td></td>
<td>OR = 0.88 CI 95%: 0.77-0.99</td>
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<tr>
<td></td>
<td>rs2236319 A&gt;G</td>
<td>$P = 0.044$</td>
<td></td>
<td>4 SNPs were nominally associated with diabetic nephropathy however the haplotype consisting of 11 SNPs within SIRT1 had a stronger association than individual SNPs</td>
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<tr>
<td></td>
<td></td>
<td>OR = 1.12 CI 95%: 1.00-1.24</td>
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<tr>
<td></td>
<td>rs10823108 G&gt;A</td>
<td>$P = 0.038$</td>
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<tr>
<td></td>
<td></td>
<td>OR = 1.12 CI 95%: 1.01-1.24</td>
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</table>
### Mitochondria and Chronic Kidney Disease

<table>
<thead>
<tr>
<th>SNP</th>
<th>Haplotype/TATAGATAGTA</th>
<th>OR</th>
<th>P Value</th>
<th>Function and Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3818292 A&gt;G</td>
<td>1.24 (P = 0.042) OR (= 1.12) (CI 95%: 1.01–1.24)</td>
<td>Catalyses the dismutation of superoxide into oxygen and hydrogen peroxide to maintain proper mitochondrial biogenesis and function</td>
<td>These SNPs were associated with incipient nephropathy and decline of eGFR in prospective study. Associated with established/advanced nephropathy in follow-up</td>
<td></td>
</tr>
<tr>
<td>rs2758329 C&gt;T</td>
<td>rs8031 A&gt;T (P = 0.005) OR (= 2.08) (CI 95%: 1.26–3.53)</td>
<td>Detoxify and reduce production of superoxide and other free radicals</td>
<td>Subjects were South Indian primarily of Dravidian origin and North Indian of Indo-European origin with T2DM for more than 10 years</td>
<td></td>
</tr>
<tr>
<td>rs4880 C&gt;T</td>
<td>(P = 0.009) OR (= 1.99) (CI 95%: 1.20–3.36) (P = 0.005) OR (= 2.16) (CI 95%: 1.28–3.72)</td>
<td>Subjects were South Indian primarily of Dravidian origin and North Indian of Indo-European origin with T2DM for more than 10 years</td>
<td></td>
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</tr>
<tr>
<td>SOD2</td>
<td>rs2758329 C&gt;T (P = 0.005) OR (= 2.08) (CI 95%: 1.26–3.53)</td>
<td>Detoxify and reduce production of superoxide and other free radicals</td>
<td>Subjects were South Indian primarily of Dravidian origin and North Indian of Indo-European origin with T2DM for more than 10 years</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- **rs3818292 A>G** is associated with incipient nephropathy and decline of eGFR in prospective study.
- **rs2758329 C>T** and **rs8031 A>T** are associated with established/advanced nephropathy in follow-up.
- **rs4880 C>T** is associated with both incipient and established/advanced nephropathy.
- **Ala9Val** and **UCP1** are associated with survival ability to comorbid complications and may be nephroprotective.
Mitochondria and Chronic Kidney Disease

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs</th>
<th>P-Value</th>
<th>OR (95% CI)</th>
<th>Function/Role</th>
<th>Population Characteristics</th>
<th>Evidence/Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala64Thr</td>
<td></td>
<td>0.015</td>
<td>2.099</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOS3 Glu298Asp</td>
<td></td>
<td>0.002</td>
<td>2.103</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GSTP1 ille105Val, A&gt;G</td>
<td></td>
<td>0.003</td>
<td>1.888</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranous Nephropathy PLA2R1 rs35771982</td>
<td></td>
<td>$1.90 \times 10^{-29}$</td>
<td>2.36 (95% CI: 2.03 to 2.74)</td>
<td>Induces cell death in a mitochondrial ROS-dependent manner which may play an important role in tumour suppression</td>
<td>The population under investigation consisted of Chinese subjects with and without idiopathic membranous nephropathy</td>
<td>Closely associated with circulating anti-PLA2R antibodies in serum as well as the expression of PLA2R in glomeruli.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.23x10^{-29}</td>
<td>2.32 (95% CI: 2.00 to 2.69)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>4.17x10^{-10}</td>
<td>2.35 (95% CI: 2.02 to 2.73)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DQA1 rs2187668</td>
<td></td>
<td>$1.11 \times 10^{-14}$</td>
<td>2.42 (95% CI: 1.93 to 3.05)</td>
<td>Evidence for localisation in mitochondria</td>
<td>Belongs to the HLA class II alpha chain paralogues and plays a central role in the immune system by presenting peptides derived from extracellular proteins.</td>
<td></td>
</tr>
<tr>
<td>IgA Nephropathy ITGAM rs7190997</td>
<td></td>
<td>$2.26 \times 10^{-19}$</td>
<td>1.22</td>
<td>Mitochondria-associated ER Membrane protein</td>
<td>Subjects were of Chinese Han ethnicity with and without IgA nephropathy</td>
<td>Alpha M beta 2 integrin is important in the adherence of neutrophils and monocytes to stimulated endothelium, and also in the phagocytosis of complement coated particles. Downregulated in diabetes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.10x10^{-13}</td>
<td>1.32</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NODAT ATF6 rs2340721</td>
<td></td>
<td>0.05</td>
<td></td>
<td>Activator of mTOR which acts as a key sensor for</td>
<td>Subjects were of Caucasian</td>
<td>This SNP has been associated with an</td>
</tr>
</tbody>
</table>
### Mitochondria and Chronic Kidney Disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs Number</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPARG</strong></td>
<td>rs1801282</td>
<td>0.02</td>
<td>8.5 (1.4–52.7)</td>
<td>Origin tested for diabetes 7, 90 and 365 days after kidney transplant increased BMI in a renal transplant cohort, pre-diabetes in a Chinese cohort, and has been found in tight linkage disequilibrium (in Caucasians) with rs2070150 which is associated with T2DM in Pima Indians</td>
<td>[226,230]</td>
</tr>
<tr>
<td><strong>PPARGC1A</strong></td>
<td>rs8192678</td>
<td>0.03</td>
<td>0.26 (0.08–0.91)</td>
<td>Regulated by mTOR and promote cell growth, proliferation, metabolism and survival. Previously associated with T2DM</td>
<td>[226,231]</td>
</tr>
<tr>
<td><strong>APOL1</strong></td>
<td>G1 allele (rs73885319 and rs60910145)</td>
<td>0.07 x 10^{-23}</td>
<td>1.1 x 10^{-39}</td>
<td>Encodes a secreted high-density lipoprotein which binds to apolipoprotein A-I. Apolipoprotein A-I is a relatively abundant plasma protein and is the major apoprotein of HDL. This essential in maintaining normal mitochondrial biogenesis and function.</td>
<td>Presence of both risk alleles vs none or only one have OR of 10.5 for FSGS risk</td>
</tr>
<tr>
<td></td>
<td>G2 allele (rs71785313)</td>
<td>4.38 x 10^{-7}</td>
<td></td>
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<tr>
<td><strong>PDSS2</strong></td>
<td>rs973457</td>
<td>0.001 OR = 2.6</td>
<td></td>
<td>Involved with coenzyme Q Synthesis which is essential for mitochondrial function</td>
<td>CoQ10 expression is decreased in FSGS independently of PDSS2 haplotype</td>
</tr>
</tbody>
</table>

Subjects were recruited from 21 US-based academic medical centers as part of the National Institutes of Health (NIH) FSGS Genetic Science}

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<table>
<thead>
<tr>
<th>Study</th>
<th>SNP</th>
<th>Effect</th>
<th>Gene</th>
<th>Allele</th>
<th>P-value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESRD</td>
<td>COQ5</td>
<td>rs1167726</td>
<td>P = 1.00×10⁻⁶</td>
<td>Essential component of ETC</td>
<td>All people included in the analysis were of white European origin and were diagnosed with T1DM before the age of 31 years</td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>COX6A1</td>
<td>rs12310837</td>
<td>P = 2.00×10⁻⁶</td>
<td></td>
<td>Associated with both diabetic kidney disease and end-stage renal disease in the discovery and in silico replication analyses subjects with T1DM</td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>GATC</td>
<td>rs2235222</td>
<td>P = 3.00×10⁻⁶</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>GATC</td>
<td>rs7137953</td>
<td>P = 3.00×10⁻⁶</td>
<td></td>
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<tr>
<td>ESRD</td>
<td>TOP1MT</td>
<td>rs724037</td>
<td>P = 2.00×10⁻³</td>
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<tr>
<td>ESRD</td>
<td>PACRG</td>
<td>rs2147653</td>
<td>P = 0.003</td>
<td></td>
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<tr>
<td>ESRD</td>
<td>APOL1</td>
<td>G1 allele (rs73885319 and rs60910145)</td>
<td>P = 1.1×10⁻³⁹</td>
<td>Encodes a secreted high density lipoprotein which binds to apolipoprotein A-I. Apolipoprotein A-I is a relatively abundant plasma protein and is the major apoprotein of HDL. This essential in maintaining normal mitochondrial biogenesis and function.</td>
<td>Self-identified African Americans with ESRD were recruited from Wake Forest– affiliated and Emory School of Medicine– affiliated outpatient hemodialysis facilities in northwestern North Carolina and Atlanta, Georgia</td>
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</tr>
<tr>
<td>ESRD</td>
<td>APOL1</td>
<td>G2 allele (rs71785313)</td>
<td>P = 8.8×10⁻¹⁸</td>
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<tr>
<td>ESRD</td>
<td>SLC22A2</td>
<td>rs316009</td>
<td>P = 0.042 OR 1.23 (95% CI: 1.02–1.48)</td>
<td>Evidence for mitochondrial localisation</td>
<td>Polyspecific organic cation transporters in the liver, kidney, intestine, and other organs are critical for elimination of many endogenous small organic cations as well as a wide array of drugs and environmental toxins. Found primarily in the kidney, where it may mediate the first step in cation</td>
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<tr>
<td>ESRD</td>
<td>SLC22A2</td>
<td>rs614226</td>
<td>P = 1.00×10⁻⁶</td>
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</table>

References:
- rs73885319 and rs60910145
- rs71785313
- rs316009
- rs12310837
- rs2235222
- rs7137953
- rs724037
- rs2147653
- rs614226
- rs316009
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<tbody>
<tr>
<td>RGMA - MCTP2</td>
<td>rs12437854</td>
<td>$P = 2.0 \times 10^{-9}$</td>
<td>OR 1.80</td>
<td>(95% CI: 1.48–2.17)</td>
<td>RGMA localises to mitochondria and may play a role as a tumor suppressor in some cancers</td>
<td>3 discovery cohorts from GENIE consortium: UK-ROI, FinnDiane and GoKinD US</td>
<td>Associated with time from T1DM diagnosis to development of macroalbuminuria.</td>
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</table>

SNP - Single Nucleotide Polymorphism ; eGFR – Estimated Glomerular filtration rate; RS – Reference SNP ID; P – P-value; OR – Odds Ratio; CI – Confidence Interval; GWAS – Genome Wide Association Study; GENIE – Genetics of Nephropathy an International Effort; UK-ROI – United Kingdom and Republic of Ireland cohort; FinnDiane – Finnish Diabetic Nephropathy Study; GoKinD – Genetics of Kidneys in Diabetes ; DNA – Deoxyribonucleic Acid; DM - Diabetes Mellitus; CKD – Chronic Kidney Disease; T2DM – Type 2 Diabetes Mellitus; DKD – Diabetic Kidney Disease; ESRD – End Stage Renal Disease; T1DM – Type 1 Diabetes Mellitus; SURGENE – Survival Genetic Nephropathy; ROS – Reactive Oxygen Species; HLA – Human Leukocyte Antigen; ER – Endoplasmic Reticulum; IgA – Immunoglobulin A; Mtor – mechanistic target of rapamycin; HDL – high-density lipoproteins ; FSGS – Focal segmental glomerulosclerosis; ETC – Electron Transport Chain; REGaTTA – Renal GeneTics TrAnsplantation
Figure 1. Internal structure of a mitochondrion including mitochondrial DNA and encoded proteins of the ETC.
Each mitochondrion (A) contains multiple copies of mitochondrial DNA (mtDNA) which encode. The mitochondrial genome (b) is a circular genome composed of 16,569 base pairs and encodes 37 genes. 14 of these genes code for proteins of the electron transport chain (c) as well as 2 ribosomal RNAs and 21 transfer RNAs required for translation of messenger RNA. The colour of the genes in (B) are matched to the colours of the ETC proteins in (C), from this it can be seen that complex 2 is encoded entirely by nuclear genes, highlighting the importance of nuclear genes to proper mitochondrial function. From the illustration in (A) it can be seen that mtDNA is often located close to stalked particles which contain the proteins of the ETC which may contribute to a higher mutation rate compared with nuclear DNA, due to ROS generated as a respiratory by-product.
Mitochondria and chronic kidney disease: a molecular update

Supplementary Table 1: Nuclear encoded genes influencing mitochondrial function and reported to be involved with chronic kidney disease

Supplementary Table 2: Examples of DNA damage and protective mechanisms
### Table 1: Nuclear encoded genes influencing mitochondrial function and reported to be involved with chronic kidney disease

<table>
<thead>
<tr>
<th>Biological Process</th>
<th>Gene</th>
<th>Physiological Role</th>
<th>Expression in Chronic Kidney Disease (CKD)</th>
<th>Pathological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial Biogenesis and Function</td>
<td>PPARγC1A</td>
<td>Regulates expression of TFAM, COX6C, COX7C, UQCRH; MCAD; NFE2L2; SIRT3; NRF1</td>
<td>Downregulated in PBMC of CKD undergoing PD compared to healthy subjects (p &lt; 0.001)[7]</td>
<td>Transcriptional coactivator. Interacts with several transcription factors to regulate mitochondrial biogenesis[1] Downregulation may be induced by ROS as a protective adaptation to reduce further ROS generation[2,3] Downregulation of PGC1α will also effect expression of downstream genes</td>
</tr>
<tr>
<td></td>
<td>NFE2L2</td>
<td>Transcription factor which acts with PPARγC1A to stimulate expression of a range of nuclear genes involved with mitochondrial biogenesis and function[4,5] Main regulator of TFAM[6]</td>
<td>Downregulated in PBMC of CKD patients undergoing PD compared to healthy subjects (p &lt; 0.001)[7]</td>
<td>Decreased expression of NRF-1 along with PGC1α will reduce expression of downstream targets of these genes and reduced mitochondrial biogenesis and OXPHOS activity in mitochondria which may be an attempt to reduce ROS production and oxidative stress[8,9]</td>
</tr>
<tr>
<td></td>
<td>TFAM</td>
<td>Expression regulated by NRF2; Regulates mitochondrial transcription and replication</td>
<td>Downregulated in PBMC of CKD undergoing peritoneal dialysis (PD) compared to healthy subjects (p &lt; 0.001)[7]</td>
<td>Reduced TFAM expression will alter mitochondrial transcription and replication machinery and reduce the mtDNA copy number[10]</td>
</tr>
<tr>
<td></td>
<td>NDUFS3, NDUFA6; NDUFA1; NDUFBI</td>
<td>Upregulated in PD compared to controls[7] Reduced activity increases oxidative stress and inflammation whereas increased expression may protect against this damage in CKD[7]</td>
<td>Increased NFE2L2 expression may protect against oxidative damage and confer anti-inflammatory properties. Mice models have also demonstrated that deletion of this gene may lead to lupus-like autoimmune nephritis[11,12] Increased expression in CKD may be an attempt to neutralise ROS production[13]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SDH5, NDUF5</td>
<td>Superoxide dismutase (SOD) catalyses the dismutation of superoxide anions to oxygen and hydrogen peroxide – an important antioxidant defence mechanism</td>
<td>Upregulated in PD compared to controls[7]</td>
<td>SOD inactivation may compromise mitochondrial function and reduce mitochondrial biogenesis and reduce antioxidant activity of mitochondria and increase ROS[14] Increased expression in CKD may be an attempt to neutralise ROS production[13]</td>
</tr>
<tr>
<td></td>
<td>UQCRH</td>
<td>Ubiquinol-Cytochrome C Reductase Hinge Protein – Complex III Subunit VIII</td>
<td>Downregulated in PBMC of CKD undergoing PD compared to healthy subjects (p &lt; 0.001)[7] Up-regulated in PBMC of CKD (stage 4–5) patients in conservative treatment and HD compared to CKD stage 2–3 and healthy subjects (p &lt; 0.002, FDR = 1%)[15]</td>
<td>Downregulation may be a protective response against increased ROS generation and chronic cellular perturbation associated with oxidative injury[16] Upregulation in PBMC may be an attempt to compensate for reduced OXPHOS activity or altered expression due to Haemodialysis treatment[17] Statistically significant difference in mtRNA levels of UQCRH between CKD 2–3 and healthy subjects[18]</td>
</tr>
<tr>
<td></td>
<td>UQCRB</td>
<td>Ubiquinol-Cytochrome C Reductase Binding Protein – Complex III Subunit VI</td>
<td>Up-regulated in PD compared to controls.</td>
<td>Upregulation in PBMC may be an attempt to compensate for reduced OXPHOS activity or altered expression due to Haemodialysis treatment[19]</td>
</tr>
<tr>
<td></td>
<td>COX6C, COX7C</td>
<td>Cytochrome c oxidase subunit 6C, subunit 7C – Subunits of complex IV</td>
<td>Downregulated in PBMC of CKD patients undergoing PD compared to healthy subjects (p &lt; 0.001) Up-regulated in PBMC of CKD (stage 4 – 5) patients in conservative treatment and haemodialysis (HD) compared to CKD stage 2 – 3 and healthy subjects (p &lt; 0.002, FDR = 1%)[15]</td>
<td>Increased expression of Complex IV may be a compensatory response to dysregulated OXPHOS in an attempt to restore the electrochemical gradient in the outer side of the inner mitochondrial membrane[20]</td>
</tr>
<tr>
<td></td>
<td>ATP5F1, ATP5S1, ATP5O</td>
<td>ATP Synthase, H+ Transporting, Mitochondrial Fo Complex Subunit E (ATP5S); Subunit F6 (ATP5S); O Subunit (ATP5O) – Subunits of Complex V</td>
<td>Up-regulated in CKD patients in conservative treatment and HD compared to healthy subjects (p &lt; 0.003, FDR = 1%)[15]</td>
<td>Increased expression of Complex V components may be a compensatory response to defective OXPHOS. This increased expression may also lead to increased ROS generation and oxidative stress further damaging mitochondria through a vicious cycle</td>
</tr>
<tr>
<td></td>
<td>MCAD</td>
<td>Medium Chain Acyl CoA Dehydrogenase</td>
<td>Downregulated in PBMC of CKD undergoing peritoneal dialysis PD compared to healthy subjects (p &lt; 0.001)[7]</td>
<td>MCAD catalyses the first step of mitochondrial fatty acid beta-oxidation and is an isodrinidize enzyme regulated by PGC1α. Downregulation of this will decrease the breakdown of fatty acid molecules in mitochondria</td>
</tr>
<tr>
<td></td>
<td>APM1</td>
<td>Codes for Apoptosis inducing factor (AIF) which is a mitochondrial flavoprotein with dual roles in redox signalling and programmed cell death[21,22]</td>
<td>Downregulated in CKD[7]</td>
<td>Partial knockdown disrupts mitochondrial bioenergetics, induces mitochondrial fusion and increases mitochondrial ATP content and leads to excess production of ROS. Complete inactivation disrupts complex 1 formation resulting in OXPHOS defects. However, expression of PGC1α and TFAM was not changed, suggesting that mitochondrial biogenesis is not altered by AIF knockdown[22]</td>
</tr>
</tbody>
</table>
| | APOE2 | Reduces SOD2 and Catalase expression[23] | Increased expression of renal risk variants G1 and G2 in various renal complications including CKD[24] | Decreased SOD2 may exacerbate mitochondrial damage by ROS[25,26] G1 and G2 risk variants associated with renal disease amongst the African Diaspora likely due to the protection against African sleeping sickness provided by these
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>CKD/variant association</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOX4</td>
<td>Main isoform of NADPH oxidase expressed in kidneys</td>
<td>Up-regulation in CKD associated with AIFM1 deletion[13,17]</td>
</tr>
<tr>
<td></td>
<td>Key source of ROS and important component in Redox signalling</td>
<td>Uncontrolled ROS generation may damage mitochondria therefore leading to further ROS production through a vicious cycle[20].</td>
</tr>
<tr>
<td>RMN01</td>
<td>Protein encoded by this gene is localized in the mitochondria and involved in mitochondrial translation.</td>
<td>Genetic variants implicated in multisystem failure due to mitochondrial dysfunction – including renal complications PD compared to controls[22].</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mutations in this gene are associated with combined oxidative phosphorylation deficiency and mitochondrial dysfunction of varying severity – Of the reported cases, seven had renal involvement additional to mitochondrial dysfunction at or earlier than 18 months of age. 3 patients with renal dysfunction where found to be homozgous for a c.1349G&gt;C, p.450Serext*32 stop-extension mutation in RMN01[22,24].</td>
</tr>
</tbody>
</table>
## Supplementary Table 2: Examples of DNA damage and protective mechanisms

<table>
<thead>
<tr>
<th>Type of Genome alteration</th>
<th>Source of damage</th>
<th>Effect on DNA</th>
<th>Repair Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative/ nitrosative stress by free radical damage</td>
<td>ROS/RNS resulting in oxidative/nitrosative stress</td>
<td>Production of 8-oxoguanine resulting in G&gt;T substitutions</td>
<td>Base excision repair, antioxidant defences and repair by HSPs</td>
</tr>
<tr>
<td>Hydrolytic Damage</td>
<td>Depurination/Depyrimidation</td>
<td>BP substitutions</td>
<td>Base excision repair</td>
</tr>
<tr>
<td>Deamination</td>
<td>Hydrolytic damage resulting in spontaneous removal of the entire amine group from a base.</td>
<td>Cytosine &gt; Uracil; Adenine &gt; Hypoxanthine; Guanine &gt; Thymine; 5-methylcytosine &gt; thymine (concentrated in CpG islands and involved with DNA methylation)</td>
<td>Base excision repair</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UV induced damage resulting in dimerization of DNA</td>
<td>Introduce local conformational changes in DNA structure and interfere with base pairing during DNA replication.</td>
</tr>
<tr>
<td>Pyrimidine dimers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylation or Bulky Adducts</td>
<td>Resulting from exposure to polycyclic aromatic hydrocarbons from atmospheric pollutants such as those present in cigarette smoke</td>
<td>Bind covalently to proteins, lipids, and guanine residues of DNA to form DNA adducts</td>
<td>Nucleotide excision repair</td>
</tr>
<tr>
<td>Single-strand breaks, small base damage</td>
<td>Genotoxic agents such as ionizing radiation, oxidizing agents, and alkylating agents</td>
<td>May lead to substitutions and DSB in DNA</td>
<td>Base excision repair</td>
</tr>
<tr>
<td>Interstrand crosslinks</td>
<td>Covalent linkage of two strands by bi-functional alkylating agents</td>
<td>Interfere with cellular metabolism, such as DNA replication and transcription, triggering cell death.</td>
<td>Homologous recombination</td>
</tr>
<tr>
<td>Double-strand breaks</td>
<td>Large DNA deletions due to damage by radiation such as UV or other genotoxic agents. May also occur when replication forks encounter SSBs.</td>
<td>Chromosome Rearrangements</td>
<td>Homologous recombination (in mid-S phase or mid G2 phase of mitosis) / Non-homologous end joining (in G0, G1 and G2 phases)</td>
</tr>
<tr>
<td>Mismatches</td>
<td>Base substitution mismatches and insertion-deletion mismatches which occur during DNA replication</td>
<td>Introduce major changes in the canonical recognition rules, and may alter structure and stability of the DNA helix</td>
<td>Mismatch Repair</td>
</tr>
<tr>
<td>Telomere attrition</td>
<td>Part of the aging process but may be expediated by smoking, obesity, stress and environmental pollutants</td>
<td>Replicative senescence, accelerated aging and reduction in tumour suppressor mechanisms.</td>
<td>Telomerase reverse transcriptase / RecQ</td>
</tr>
</tbody>
</table>

Supplementary References


16. Kokoszka JE, Coskun P, Esposito LA, Wallace DC. Increased mitochondrial oxidative stress in the SOD2 (+/-) mouse results in the age-related decline of mitochondrial function culminating in


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