Cytokine gene polymorphism and progression of renal and cardiovascular diseases

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Cytokines are important modulators of inflammation. The balance between pro- and anti-inflammatory cytokines determines whether the intensity of inflammatory response is within physiological limits or in the pathological range. The cytokine network is highly complex, containing interactive cascades of gene activation and suppression. Both chronic kidney disease (CKD) and end-stage renal disease (ESRD) are characterized by elevated levels of proinflammatory cytokines and markers of inflammation. Cytokines may modulate the risk for progression of renal disease and the susceptibility to cardiovascular disease (CVD). Polymorphisms of cytokine genes may influence gene transcription and cytokine secretion and thereby modulate the risk of progression of renal and CVDs. The observed inconsistencies in the data regarding associations between single-nucleotide gene polymorphisms (SNPs) and their presumed phenotypic expression emphasize the need to recognize several conceptual and methodological aspects such as haplotypic rather than single SNP variations and the influence of pathway genes with synergistic or antagonistic effects that ultimately determine the phenotype. It is conceivable that when a patient with a high-risk cytokine genotype develops CKD, the risk for CVD is increased. Early interventions in CKD patients with high-risk genotypes may slow the progression of renal disease and also decrease CV mortality and morbidity.

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Cytokines are soluble polypeptides acting as important humoral modulators in immunoregulation, hematopoiesis, and the inflammatory cascade. Cytokines act in a highly complex coordinated network with autocrine and paracrine attributes. Many cytokines appear to be pleiotropic in their actions, with considerable overlap and redundancy between the function of individual cytokines. Patients with chronic kidney disease (CKD) have markedly elevated levels of cytokines. Although all available evidence indicates upregulation of proinflammatory cytokine activity in CKD, the etiology of this is largely unknown. The emerging evidence suggests that cytokines may play a vital regulatory role in the initiation and progression of CKD and cardiovascular diseases (CVDs).

THE EPIDEMIC OF CKD

The incidence and prevalence of CKD in the United States is rapidly increasing; nearly 3% of the US adult population was noted to have an elevated serum creatinine in the Third National Health and Nutrition Examination Survey (NHANES III).[1] The burden of morbidity and mortality from CKD derives not only from the progression to end-stage renal disease (ESRD) but also the associated risk of CVD that stems from the cumulative effects of multiple risk factors from the early stages of CKD. CKD has profound economic implications for the health-care system, as it is consistently associated with increased risk of hospitalization, CV events, and death. Although some traditional ('Framingham') risk factors such as older age, male gender, Caucasian race, family history, and cigarette smoking, contribute to disease among patients with CKD, just as they do in the general population, other risk factors, including hypertension, diabetes/insulin resistance, and dyslipidemia occur with a higher prevalence and/or severity in patients with CKD than in the general population.

THE ROLE OF INFLAMMATION IN CVD AND CKD

Epidemiological studies have demonstrated that inflammation is an important link between CKD and CVD.

Preliminary evidence suggests a fundamental role for innate and adaptive immunity in the pathogenesis of CVD as well as progression of CKD. Patients with CKD have higher circulating levels of cytokines and dysregulated cytokine metabolism, leading to elevated levels of acute phase proteins. The role of chronic inflammation in atherosclerotic coronary artery disease (CAD), stroke, and peripheral vascular disease is well established; elevated C-reactive protein (CRP) is a proven marker for atherosclerosis and CV mortality in the general population as well as among patients with CKD/ESRD. Studies in animal models have highlighted the fundamental role for innate and adaptive immunity in all stages of atherosclerosis.[2,3] Toll-like receptors recognize pathogen-associated molecular patterns. They are also activated by endogenous pro-atherosclerotic ligands such as oxidized low-density lipoprotein and CRP. Activation of Toll-like receptors also induces cytokine effectors, including tumor necrosis factor-alpha and -beta (TNF-α and -β), interleukin-1 beta (IL-1β), IL-1 receptor antagonist (IL-1Ra), and IL-6. Cytokine receptors (TNF receptors – TNFR1 and R2, TNFRSF1A and 1B; IL-1 receptors – IL-1R1 and R2; IL6 receptors – IL-6R and IL-6ST), in turn, modulate bioactivity of their respective cytokines.[4] Toll-like receptors and cytokines activate transcription factors – mitogen-activated protein kinases, p38, and nuclear factor-κB that in turn modulate transcription of cytokine effector molecules. Vascular endothelial, smooth muscle, and mononuclear cells are the source of a number of these cytokines and growth factors, including IL-1, TNF-α, transforming growth factor-beta (TGF-β), platelet-derived growth factor and fibroblast growth factor.[5] These molecules potentially modulate the phenotypic expression of neighboring cells in a paracrine manner and themselves in an autocrine manner, including transformation of smooth muscle cells from contractile to synthetic phenotype, leading to cell proliferation.[6]

In CKD, once a critical proportion of nephron mass is lost, 'nonspecific' glomerular and tubulointerstitial scarring results, which is characteristic of all forms of CKD. The final common pathway of mechanisms of progression of renal disease includes intraglomerular hypertension, hyperfiltration in the remnant glomeruli, systemic hypertension, poor glycemic control (in diabetics), mesangial, endothelial, and epithelial injury from protein and macromolecular trafficking, and hyperphosphatemia. Progressive and relentless glomerular injury is also facilitated by inflammatory and profibrotic cytokines.[7–9] Cytokines modulate the glomerular response to infectious, dietary, and environmental antigens, and hence influence the progression of CKD.[10] In the kidney, cytokines induce (a) resident cells to proliferate,[11] (b) aberrant matrix metabolism,[12,13] (c) procoagulant activity of endothelium,[14] (d) reactive oxygen/nitrogen species,[15] and expression of (e) adhesion receptors,[16] bioactive lipids,[17] and metalloproteinase.[18] These molecules may be the effectors through which the renin–angiotensin system and hemodynamic factors mediate their actions.[9] Increasing levels of angiotensin II in CKD may upregulate the expression of growth factors and cytokines, such as TGF-β1, TNF-α, osteopontin, vascular cell adhesion molecule-1, nuclear factor-κB, platelet-derived growth factor, fibroblast growth factor, and insulin-like growth factor.[19–21] Angiotensin II also stimulates oxidative stress, which upregulates the expression of adhesion molecules, chemo-attractant compounds, and cytokines. Nuclear factor-κB stimulates the angiotensinogen gene, and is in turn activated by angiotensin II providing an autocrine reinforcing loop.[22]

GENETIC RISK IN CKD AND CVD

The progression of renal disease varies not only with the etiology of underlying renal disease but also among patients with same type of renal disease.[23] Familial clustering of CKD and ESRD has been appreciated, indicating the presence of a genetic component. Freedman and co-workers[24] observed heritability of ESRD among large African-American families. Among index cases with type II diabetes mellitus and ESRD, 37% reported a close relative with ESRD; in contrast, only 7% of age- and gender-matched individuals with type II diabetes mellitus without nephropathy reported ESRD in close relatives. Likewise, family history is an important independent risk factor for CAD and many risk factors for CVD, such as lipid levels, hypertension, obesity, diabetes, and the metabolic syndrome, show significant heritability in family- and population-based epidemiological studies. Genetic risk may also play a role in the disparities in CVD-related mortality among ESRD patients from different countries. A recent ecological study highlighted the strong correlation to underlying rates of CVD in the respective general populations; indeed, gene polymorphisms may explain a large part of the ethnic and mortality differences among dialysis populations (see Table 1 for a glossary of common terms).[25]

Emerging studies of complex human disease provide compelling evidence for multilocus effects on common traits.[26] The spectrum of CKD and ESRD, and their complications such as CVD are typical examples of complex disorders, which in contrast to Mendelian or monogenic disorders, demonstrate a phenotype controlled by multiple genes (polygenic, locus heterogeneity) and multiple biological pathways. The contribution of each gene is small (low genotypic relative risk). Phenotypic expression is characterized by variable severity and/or definition and context dependency, and is often modified by gene–environment interactions and gene–gene interactions (epistasis). The rate of progression of renal disease and propensity to CVD is seen to vary widely among patients with CKD, even when corrected for ethnicity, the etiology of renal disease, and comorbid conditions. Preliminary evidence suggests that cytokine genes influence transcription and function as a result of natural variations in their sequences (i.e. single-nucleotide polymorphisms (SNPs)). They are attractive as candidate genes modulating the risk for both CKD and its complications. Candidate gene association studies are a powerful approach for the identification of common genetic

Table 1 | Glossary of common terms

Allele: Alternative form of a genetic locus; a single allele for each locus is inherited from each parent.

Candidate gene: A gene located in a chromosome region suspected of being involved in a disease. Methods to identify candidate genes include basic science studies, identifying DNA sequences conserved across species, human genetics, or genome-wide analyses.

Epigenetics: Change in the pattern of gene expression that is mediated by mechanism other than alterations in the primary nucleotide sequence of gene, which may or may not be heritable.

Exon: The protein-coding DNA sequence of a gene.

Genome: All the genetic material in the chromosomes of a particular organism; its size is generally given as its total number of base pairs.

Genotype: The genetic constitution of an organism, as distinguished from its physical appearance (its phenotype).

Haplotype: A way of denoting the collective genotype of a number of closely linked loci on a chromosome. A set of genetic variants that are inherited together. Polymorphisms that are co-inherited more often than by chance alone are in linkage disequilibrium. Haplotype blocks may include many individual polymorphisms in high-linkage disequilibrium; as a result, establishing genotype at any single polymorphic site with such a block may establish genotypes at linked sites within the block. Individual SNPs that can be used to establish genotype within a haplotype block are termed tag-SNPs.

Intron: DNA sequence that interrupts the protein-coding sequence of a gene; an intron is transcribed into RNA but is cut out of the message before it is translated into protein.

Linkage disequilibrium: Where alleles occur together more often than can be accounted for by chance. Indicates that the two alleles are physically close on the DNA strand.

Phenotype: The measurable physical characteristics of an organism or the presence of a disease that may or may not be genetic. These may derive from genotype, environment, or the combination. Organisms with the same phenotype can have different genotypes.

Polymorphisms: Difference in DNA sequence among individuals that may underlie differences in health. Genetic variations occurring in more than 1% of a population would be considered useful polymorphisms for genetic linkage analysis. Polymorphisms can be in coding regions or more commonly, in non-coding regions, and often vary by ethnicity. The most common type of polymorphism is a change in one nucleotide (base pair) in a DNA sequence, referred to as an SNP. Other polymorphisms are insertion and deletion of multiple sequential nucleotides ('indels'); variable numbers of repeats, such as doublets or triplets; or large-scale duplications or deletions. Although some genetic variants are known to alter protein abundance or function, the functional consequences of most polymorphisms are unknown.

SNP: DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered.

tag-SNPs: These are maximally informative SNPs that characterize common haplotypes.

SNP, single-nucleotide polymorphism.
Source: Department of Energy Human Genome Program genomics glossary (http://www.ornl.gov/sci/techresources/Human_Genome/glossary/glossary_s.shtml).

variants involved in common diseases. This is especially true of studies in the context of CKD and ESRD, where the existence of multiple physiological trade-offs and reverse causality give rise to extensive confounding influences. The concept of Mendelian randomization, the random assortment of alleles from parent to offspring at gamete formation, could potentially strengthen such designs.

CYTOKINE GENE POLYMORPHISMS AND THE COMPLEXITY OF CYTOKINE GENE REGULATION

Stimulation of human blood samples with bacterial lipopolysaccharide shows large interindividual variations in the production of cytokines, a likely function of genetic variability (Table 2). About 11 million SNPs with minor allele frequencies of at least 1% are estimated to exist in the human genome. Fewer SNPs, perhaps 250 000, are thought to have functional significance because they occur in coding regions, splice junctions, and promoter regions. Several allelic polymorphisms of cytokine genes that regulate gene transcription are of demonstrable clinical significance (http://www.nanea.dk/cytokinesnps/). Several twin studies have also confirmed significant heritability in circulating cytokine and CRP levels.[38,39]

The existence of complex systems that make up cytokine networks makes the elucidation of cytokine-gene effects in complex diseases a significant challenge. The possibility that single genes, with small effects, could contribute substantially to disease through interaction with other genes and environment has been the conceptual breakthrough facilitating the analysis of genetic data in such settings. This approach has given rise to the need to examine the role of individual genes in the context of other pathway genes with synergistic or antagonistic effects. It has also underlined the need to know more comprehensively the complete genetic variation within a candidate gene and the structure of linkage disequilibrium in the region (see next section on the concept of haplotype variation). Failure of replication has been a frequent obstacle vitiating progress in the field; replication validity has been emphasized as a requirement toward the burden of proof for gene–disease association studies, but has been difficult to satisfy consistently, given the multiplicity of variables, interactions, and inputs within these systems.

Table 2 | Simplified summary of the putative effects of cytokine gene polymorphisms on relative levels of cytokine expression as determined by transcription and/or secretion of gene product

	Transcription/secretion by individual genotype		
Cytokine	Low	Intermediate	High
IL-1β[27,28]	−511 CC	−511 CT	−511 TT
	+3953 CC	+3953 TC	+3953 TT
IL-1 Ra[29,30]	—	—	Allele 2
IL-6[31,32]	−174 CC	−174 GC	−174 GG
	−572 GG	−572 CG	−572 CC
IL-10[33,34]	−1082 AA	−1082 GA	−1082 GG
TNF-α[35,36]	−308 GG	−308 GA	−308 AA
	−238 AA	−238 GA	−238 GG
	—	—	TNFd3 allele
TGF-β[37]	+869 CC	+869 TC	+869 TT
	+915 CC	+915 GC	+915 GG

IL, interleukin; IL-1Ra, IL-1 receptor antagonist; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha.

Other caveats that must be applied to these studies include adequate consideration of confounding and interactive variables and intermediate phenotypes and the demonstration that alleles exist in Hardy–Weinberg equilibrium. Without these stipulations, reports of both positive and negative associations must be interpreted with caution.

Ongoing ancillary studies to Chronic Renal Insufficiency Cohort (CRIC) Study[40] are examining the role of inflammatory gene networks, upstream activators of cytokine signaling, and cytokine signal transduction/and key inflammatory transcription factor pathways in the progression of renal disease and CVD. Results from this exciting study are expected by early 2008.

CONCEPT OF HAPLOTYPE

Considering the inconsistencies in the characterization of associations between SNPs and intermediate and clinical phenotypes, there is emerging interest in haplotypes (http://www.hapmap.org). Polymorphisms do not exist in isolation, and it may be the combination of base changes at several sites along the gene, that is, the haplotype that influences the function. Blocks of sequences in the same chromosome tend to be inherited together, a phenomenon known as linkage disequilibrium. Such groups of alleles that are rarely separated by recombination are known as haplotypes. Haplotypes, rather than single SNPs, may exert a concerted effect on the phenotype of interest. Inference of common haplotypes from genotypic information derived from tag-SNPs may capture a large proportion of the genetic variation across sizable regions. Thus, selection of the maximally informative set of common tag-SNP set can comprehensively interrogate for main effects from common functional variation.[41] The pertinent tag-SNPs may vary between racial/ethnic groups. Several algorithms exist that help identify the most appropriate tag-SNPs that will maximize efficiency of study resources (http://pga.gs.washington.edu). Commonly, the most suitable tag-SNPs are those that (a) overlap between different race and ethnic groups, (b) overlap with the putatively functional SNPs under study, (c) represent unique variants for which genotyping assays are readily available; an ordered preference for non-synonymous SNPs, SNPs occurring in the promoter or 3-untranslated regions, intronic and synonymous SNPs would be of greater utility and (d) are repeat polymorphisms, which are highly informative for haplotype construction.

A brief outline of most relevant cytokine gene polymorphism and their relationship to progression of renal and CVDs is discussed below (Table 3). Gene association studies in progressive kidney disease have not been as plentiful as those in CVD.

IL-1 AND IL-1RA

IL-1 family consists of two proinflammatory cytokines, IL-1α and IL-1β, and a naturally occurring anti-inflammatory agent, the IL-1Ra. The balance between IL-1 and IL-1Ra in local tissues plays an important role in the susceptibility to, and severity of many diseases. Plasma IL-1 and IL-1Ra have been shown to predict CV outcome and mortality in ESRD. These three genes of the *IL-1* complex map to the 430 kb region on the long arm of chromosome 2. The *IL-1β* gene has two base exchange (C→T) polymorphic sites at −511 and +3953.[50] *IL-1Ra* gene (IL-1RN) contains a variable number of tandem repeat polymorphisms in intron 2. IL-1RN allele 2, corresponding to a 2 U repeat of 86 bp, is associated with increased production of IL-1β.[27] Polymorphisms of *IL-1β* and *IL-1RN* have also been associated with hypertension, atherosclerosis, CAD, and progression of renal disease.[42,29]

INTERLEUKIN-6

IL-6 is the one of the most extensively studied effectors in the inflammatory cascade. Elevated circulating levels have been linked to malnutrition, hypertension, left ventricular hypertrophy, atherosclerosis, and CV mortality in ESRD patients[51] (Figure 1). IL-6 is also an autocrine growth factor for the mesangium. The human *IL-6* gene is located at chromosome 7p21, and consists of five exons and four introns. *IL-6* has several polymorphisms in the promoter region −174 G→C, −634 C→G, −572 G→C, and −597 G→A. The promoter region at position −373 is a polymorphic A_nT_n tract with six alleles.[52] The −597 G→A and −174 G→C polymorphisms are in strong allelic association and may have an additive effect on plasma IL-6 level.[31] Different haplotypes (−597 G→A/−572 G→C/−174 G→C) may determine the transcription levels of the *IL-6* gene. Associations between IL-6 gene polymorphisms (−597 G→A and −174 G→C) and CRP concentration, peripheral vascular disease, CAD, left ventricular hypertrophy, severity of stroke cardiovascular mortality,[45] and progression of diabetic renal disease[44] have been reported.

INTERLEUKIN-10

IL-10 attenuates the inflammatory response. Decreased production of IL-10 is associated with increased CRP and

Table 3 | Cytokine gene polymorphisms known to be related to progression and severity of renal and cardiovascular diseases

Authors	Genotype	Clinical significance
IL-1		
Amoli *et al.*[28]	−511 C/T	Severity of renal involvement in Henoch-Schonlein purpura
IL-1Ra		
Blakemore *et al.*[29]	IL1RN*2	Development of diabetic nephropathy in type I and type II diabetes
Shu *et al.*[42,43]	IL2RN*2	Progression in IgA nephropathy and risk for ESRD
IL-6		
Kitamura *et al.*[44]	−634 C/G	Progression of diabetic nephropathy
Liu *et al.*[45]	−174 G/C	Risk for CVD among dialysis patients
Losito *et al.*[32]	−174 G/C	LVH in hemodialysis patients, especially those with diabetes
TGF-β		
Khalil *et al.*[46]	Arg[25]	Associated with severity of proteinuria and glomerulosclerosis
Sato *et al.*[47]	−509 CC and 869 CC	Heavy proteinuria and mesangial cell proliferation
Rao *et al.*[48]	G/C substitution at codon 25	Risk for prevalent vascular disease, new onset cardiac morbidity and cardiac mortality in HD patients
TNF-α		
Thibaudin *et al.*[49]	−308 G/A TNFd2 allele	Susceptibility to idiopathic membranous nephropathy
IL-10		
Girndt *et al.*[33]	−1082A*	Lower production of IL-10 and increased CV morbidity

CVD, cardiovascular disease; ESRD, end-stage renal disease; HD, hemodialysis; IL, interleukin; LVH, left ventricular hypertrophy.

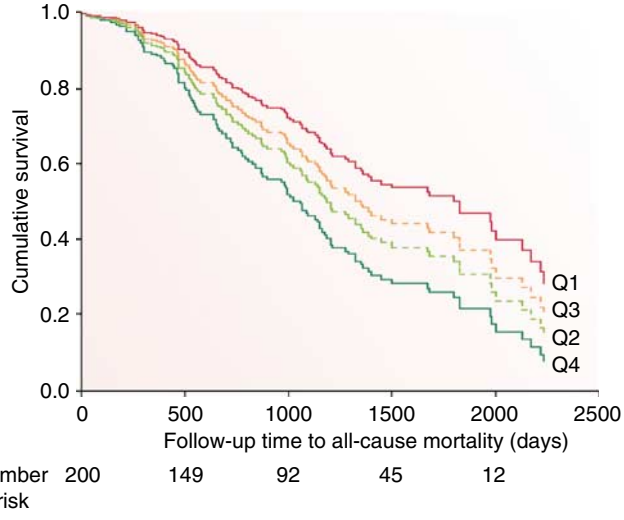

Figure 1 | Results from a study of hemodialysis patients exemplifying the association between cytokine levels and survival. The figure shows the adjusted event-free survival by quartiles of baseline plasma IL-6 levels for all-cause mortality in ESRD patients. Patients in the highest quartile of baseline plasma Il-6 level had an adjusted hazard for all-cause mortality of 1.85 times that of lowest reference quartile (from Rao *et al.*[51]).

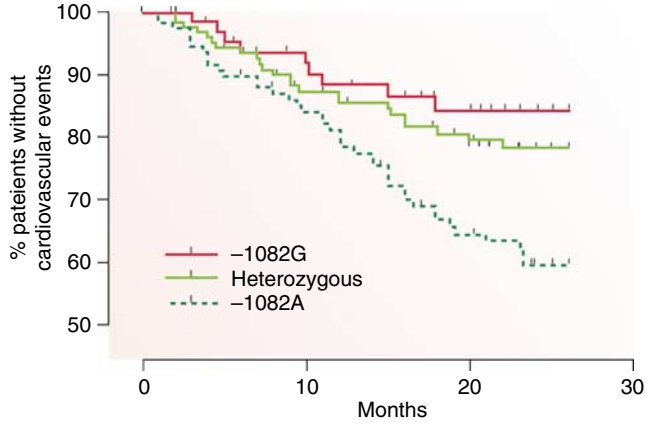

Figure 2 | Results from a study of hemodialysis patients exemplifying the association between cytokine gene polymorphisms and survival. The figure shows the cardiovascular event-free survival in ESRD patients with IL-10 gene polymorphism at position −1082. Patients homozygous for −1082A had a significantly shorter event-free survival compared to those with −1082G genotype ($P = 0.004$, all three patient groups) (from Girndt *et al.*[33]).

higher CV mortality. The *IL-10* gene is located on chromosome 1 at 1q31–32 and is composed of five exons. IL-10 gene has SNPs at positions −592 C→A, −819 C→T, and −1082 G→A as well as two CA repeat polymorphisms, IL-10 G and IL-10 A approximately at positions −1200 and −4000.[53] *IL-10* low producer genotype (−1082 AA) is associated with increased CV mortality in ESRD patients[33] (Figure 2).

TUMOR NECROSIS FACTOR-α

TNF-α is produced early in the inflammatory reaction. Increased TNF-α level is associated with metabolic syndrome, CAD, left ventricular hypertrophy, and congestive cardiac failure. TNF-α is upregulated in progressive renal disease, and plays a role in the recruitment of inflammatory cells in response to glomerular injury.[54] The *TNF-α* gene is located on chromosome 6p and is highly polymorphic.[55] Known variants in the promoter region occur at positions −1031, −863, −857, −851, −419, −376, −308, −238, −163, and

−49 relative to the transcription start site and also +488 in the intron. Most of these SNPs are G→A substitution, with the exception of −419 (G→C), −851 (C→T), 857 (C→A), −863 (C→A), and −1031 (T→C). *TNFd* microsatellite polymorphism (*TNFd*1–*d*7) consists of various dinucleotide repeats (GA) located 8 kb downstream the *TNF-α* gene.[56] Polymorphisms of *TNF-α* gene at −308 and −238 positions are related to metabolic syndrome, CAD, and stroke.[57] Recently, investigators reported that SNP at position −308 in the promoter region and *TNFd*2 allele are associated with susceptibility to idiopathic membranous nephropathy, but did not predict disease progression.[58]

TRANSFORMING GROWTH FACTOR-β

TGF-β has anti-atherogenic and anti-inflammatory properties. Overproduction of TGF-β has been linked to hypertension, left ventricular hypertrophy, vascular remodeling, and progressive renal disease. Increased circulating level of TGF-β may accelerate the deterioration of renal function by accumulation of matrix proteins, interstitial fibrosis, and mesangial expansion. Seven polymorphisms have been identified in the *TGF-β* gene, including two in the signal peptide sequence Leu^{10}→Pro (+869 T→C) and Arg^{25}→Pro (+915 G→C).[59] The proline allele at codon 10 (Pro^{10}) is associated with higher levels of TGF-β mRNA and protein. The codon 25 G/G genotype is associated with higher plasma levels of TGF-β than the G/C genotype. TGF-β codon 10 and codon 25 genotypes have been shown to be related to CAD, hypertension, and myocardial infarction.[59] Rao *et al.*[48] demonstrated that the G/C substitution at codon 25 is associated with an increased risk for prevalent vascular disease and new onset cardiac morbidity, and mortality in ESRD patients.

CACHEXIA, INFLAMMATION AND CVD

Malnutrition and cachexia coexist with inflammation in CKD. A number of investigators have demonstrated that inflammation may play a key role in the pathogenesis of protein energy wasting in CKD. Plasma levels of pro-inflammatory cytokines have been shown to be inversely related to serum albumin and muscle mass. Prevalence and severity of the malnutrition inflammation atherosclerosis syndrome is strongly associated with CVD and all-cause mortality in patients with ESRD.[60] SNPs in the promoter region of the proinflammatory cytokines IL-6 and TNF-α, and the regulatory monokine IL-10, show a strong association with indices of comorbidity and nutritional markers in ESRD patients.[35] Interestingly, some of the genes regulating skeletal muscle mass are also associated with insulin resistance, inflammation pathway, and cardiac morphology.[61]

OTHER STRUCTURAL VARIATIONS IN THE GENOME

Emerging role of epigenetics

Epigenetic alterations are potentially reversible changes in genetic material leading to alterations in gene expression. The best-known epigenetic signal is DNA methylation, which is frequently associated with transcriptional silencing of genes. Global hypomethylation may promote genomic instability. DNA hypermethylation may contribute to upregulation of atherosclerosis-susceptible genes and downregulation of atherosclerosis-protective genes and affect genes that mediate progression of renal disease. Epigenetic alterations in monocarboxylate transporter and estrogen receptor α genes that alter inflammatory response and induce phenotypic transition of smooth muscle cells have been described. Change in methylation status can modulate B- and T-cell differentiation and also production of cytokines.[62]

Copy-number variants

These are gains and losses of DNA sequences of >1 kb that may include genes resulting in differential levels of gene expression and accounting for a significant proportion of normal phenotypic variation. A recently described example of a segmental duplication encompasses the gene encoding *CCL3*L1, a potent HIV-1 suppressive chemokine and ligand for the HIV co-receptor CCR5, that influences the susceptibility to AIDS.[63]

WHY IS STUDYING CYTOKINE GENE POLYMORPHISM IMPORTANT?

The rationale for studying cytokine gene polymorphism and their inferred haplotypes are to (a) understand the cause for interindividual variation in inflammatory response, deterioration of renal function, and progression of CVD, (b) identify CKD patients at high risk for susceptibility, severity, and poor clinical outcomes, (c) develop novel strategies to prevent or delay the disease process, and (d) enhance the understanding of the etiopathogenesis of increased CVD in patients with CKD. One often overlooked aspect of cytokine gene–disease association studies is that the cytokine network is highly complex, containing interactive cascades of gene activation and suppression. Therefore, individual polymorphisms in cytokine genes association may be non-informative, whereas specific combinations of cytokine genotypes or the activation of specific cytokines in a given context might predispose to disease susceptibility or outcome. An example is the West of Scotland Coronary Prevention Study (WOSCOPS),[64] where there was no significant evidence of higher risk associated with the *−174CC IL*-6 genotype compared with the GG+GC group in the placebo arm. However, in the pravastatin-treated group, CC homozygotes had a significantly lower risk of CVD compared with the GG+GC placebo group, suggesting that the early intervention was most effective among patients with high-risk genotypes. Thus, it is reasonable to speculate that early interventions in CKD patients with high-risk genotypes might slow the progression of renal disease and also decrease CV mortality and morbidity.

To summarize, the rates of progression of CKD and CVD show wide interindividual variation, even when corrected for potential confounding influences. Cytokines, interacting with environmental and traditional risk factors, could modulate

the risk for progression of renal disease and the susceptibility to and/or progression of CVD. Polymorphisms of cytokine genes may influence the expression of gene and their gene products. Those SNPs and their inferred haplotypes that promote increased transcription of proinflammatory cytokines may be associated with risk for and also progression of renal and CVDs. Identification of patients carrying high-risk genotypes may allow early and aggressive interventions to be aimed at appropriate target populations.

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