Mini Review

Strategies for investigating the genetics of chronic kidney disease

Abstract

This short review describes the strategies employed for investigating genetic variation in chronic kidney disease as well as highlighting potential shortfalls that should be overcome in future studies.

Introduction

Since the 1980s, there have been more than 3000 research papers published on the genetics of chronic kidney disease (CKD) [1]. Initially, these had concentrated on Mendelian patterns of inheritance to understand the pathophysiology of inherited diseases such as adult polycystic kidney disease. The mutations in genes PKD1 and PKD2 have led to more informed genetic counselling regarding the progression of their disease as well as the pathways leading to this disease [2], and other familial mutations have been identified when investigating other inheritable congenital kidney diseases resulting from single gene disorders, especially in children.

Candidate gene associations in CKD

Over the last decade, single gene studies have focused on biological plausible candidate gene association with kidney disease. These studies were often underpowered with inclusion of multiple ethnicities in cohort studies, with little attempt at replication and correction for multiple confounders. However, as the 1000 genome project has recently confirmed, there is a large genomic discrepancy depending on your ethnicity and ancestry [3]. One method of counter-acting these issues is genome-wide linkage studies that combine the above two strategies, by examining large genomic regions in family-based collections of DNA to associate transmission of genetic variants to the development of CKD. Conditions such as CKD are likely to be more complex with many potential pathways and environmental changes leading to its development and progression. The ability to find enough numbers of related individuals with an underlying condition to track genetic change across multiple generations remains a barrier to success for this method.

Single Nucleotide Polymorphisms

The most common type of genomic variation is Single Nucleotide Polymorphism (SNP) that must occur in over 1% of the population to be called a SNP and has the possibility of two alleles at a given site (biallelic) [4]. Other types of polymorphisms include insertion/deletion or copy number variation of the number of nucleotides. This has led to genome-wide association studies (GWAS) into CKD with the advantage of examining millions of SNPs in unrelated affected individuals compared to controls. A GWAS typically involves using tag SNPs that are SNPs that have been ‘aggregated’ from haplotypes of nearby SNPs or SNPs that have been inherited together. GWAS will allow the identification of common SNPs that associate with disease, however may miss less common SNPs that may relate to important changes in CKD and especially in a subset of this large population. In all genomic investigations, it is paramount that the correct control group is identified and that patients are phenotyped correctly. SNPs identified may infer changes to RNA thus affecting the protein function upon translation (non-synonymous) but may have small effect size to the overall condition being investigated such as CKD. To validate SNPs found by GWAS, replication studies should be performed of the single gene variant identified as well as performing functional analyses using transcripts of tissue or other samples from patients by performing an expression as a quantitative trait locus analysis (eQTL). This could either be a transcript encoded by a nearby to SNP gene (cis-eQTL) or transcript away from that SNP with alteration of its abundance or function, either on the same or a different chromosome (trans-eQTL) [5]. A functional context of the SNP effect can be elucidated using tissue specific transcripts such as a renal biopsy sample and possible pathways to the phenotype being studied. To further examine a functional context to the associated SNP, animal models using knockouts of gene and inducing the phenotype desired can further elucidate possible pathways and therapeutic interventions. Another high-output genomic advance is the use of next generation sequencing that allows investigation of a person’s whole exome that contains the protein-coding...
part of the genome, 1–2% of the human genome, or whole genome sequencing [6]. This technique is utilised especially when examining tissue or a subgroup of cells from tissue. The advantage of this technique is the allowance to find novel mutations with phenotype association, however also proposes the challenge of interpreting large volumes of data.

**Genome wide association studies in CKD**

The phenotypes studied in GWASs in CKD have tended to focus on estimated glomerular filtration rate (eGFR), CKD progression, proteinuria and renal disease prevalence, rather than measures of cardiovascular disease or mortality.

Examples of success in GWAS association with renal disease, using the above strategies comes from the CKDGen study group who performed a meta-analysis of 20 GWASs. This included over 67000 patients studied of European ancestry who had biopsies of either their native or transplant kidney. The analysis was from population-based studies rather than case-control and examined the glomerular and tubulointerstitial gene expression in relation to declining eGFR. They had not only found 13 new loci related to kidney function and serum creatinine secretion but also found vascular endothelial growth factor A gene expression in both renal compartments with strong enrichment for the hypoxia signalling pathway [7]. GWASs have also been able to confirm laboratory findings even in small populations of disease such as in membranous nephropathy. In a French cohort of just 75 cases, the phospholipase A, receptor (PLA2R) 1 gene was found to significantly associate with membranous nephropathy [8], after previous confirmation of serological M-type PLA2R autoantibody could be found in 70% of cases to differentiate between primary and secondary membranous nephropathy which in turn, has marked therapeutic implications [9]. Subsequent investigation has revealed an odds-ratio (OR) of 2.00 with the presence of the at risk SNP genotype for PLA2R1 despite being located within the first intron (non-coding) part of the gene [10]. GWASs have also been able to confirm that the pathogenesis of disease states such as antineutrophil cytoplasmic antibody associated vasculitis has a genetic component and the subsets of this condition granulomatosis with polyangiitis and microscopic polyangiitis show genetic distinctions and thus are distinct autoimmune syndromes [11].

**Epigenetics**

As well as inherited genetic influence on outcomes and prevalence in CKD, epigenetics is a field that is helping researchers to understand the influence that environmental factors have on the human genome and thus disease presentation and variance amongst individuals. This has been observed even in phenotypic discordant monozygotic twins (with identical DNA sequence) to explain their differences [12]. There are 3 main epigenetic changes that occur, namely chromatin modification, RNA interference and DNA methylation. However, these can occur at the same time, thus unravelling their effects upon gene regulation is challenging. To add to this complexity, different cell types will express different epigenetic changes or epigenomes, thus performing epigenome-wide association studies in CKD are fraught with difficulty to understand their meaning, even if they are from the same tissue such as from kidney biopsies, due to the many cell types in this organ. Chromatin modification is thought to effect gene expression by changing DNA availability to transcriptional machinery that can result in chromatin states of transcription inactivity or activity (termed euchromatin) by histone acetylation, methylation, phosphorylation and ubiquitination. RNA interference results in impediment of translation efficiency of mRNA to protein. RNA interference can occur in short non-coding RNA (microRNAs) or long noncoding RNA and has been shown to skew the female X-chromosome in renal transplantation that is associated with a reduced allograft survival if donor female X-chromosome is skewed [13]. DNA methylation occurs at sites where a methyl group is enzymatically added to the phosphate that links cytosine to guanine (CpG site). This can alter the function of the gene by more frequently silencing the gene, but also by activation via inhibiting co-factors or microRNA that repress transcription [1].

**Conclusion**

However, despite these strategies for examining genetics of CKD, future studies will require to be more robust in phenotyping the cohort testing, and ensuring that the most clinical relevant and patient reported relevant outcomes are studied rather than just progression in kidney function that past studies have suffered from.

**References**


