Kallikreins: unravelling the genetics of autoimmune glomerulonephritis*

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Human disease gene discovery has entered a new era where genome-wide associations (GWA), and high throughput sequencing technologies are providing a first survey of the complex genetic architecture of common diseases. Immune-mediated glomerulonephritis, a shared pathological feature of systemic lupus erythematosus (SLE), systemic vasculitis and Goodpasture syndrome, displays a strong genetic component and, unlike other autoimmune diseases, GWA studies describing genetic variation associated with immune-mediated glomerulonephritis have not been yet reported. Gene identification for autoimmune glomerulonephritis has benefited, on the other hand, from the successful use of experimental rodent models as they provide an unparalleled resource for the genetic investigation of autoimmunity characterized by end-organ kidney damage.

The recent study published in Journal of Clinical Investigation by Liu et al. is an excellent example of the use of rodent models for positional cloning followed by association studies in human lupus nephritis [1]. Transcriptome analysis of the renal cortex of anti-GBM antibody-induced glomerulonephritis (nephrotoxic nephritis—NTN) in NTN-resistant and NTN-susceptible strains allowed the identification of a largely down-regulated gene family, the kallikreins (Klk) mapping to chromosome 7. Klk gene expression, protein amount and enzyme activity were markedly reduced following NTN induction in susceptible strains of mice such as DBA/1, 129/SvJ and NZW, and the pharmacological inhibition of bradykinin receptor B2, the biological target of Klk and bradykinins, exacerbated glomerulonephritis in NTN-resistant BALB/c mice, suggesting a protective role for the activity of these serine esterases in murine nephritis.

Genetic investigation of complex traits in rodent models aims to establish linkage of a given trait to a discrete chromosomal location in order to identify quantitative trait loci (QTL) in a segregating population. A QTL is a discrete chromosomal region controlling a quantitative trait such as clinical measurements (e.g. proteinuria, blood urea nitrogen) or a cell phenotype (e.g. T-cell activation). In general, QTLs correspond to relatively large chromosomal segments that may contain hundreds of genes. Finding the causative variant often requires specific genetic tools such as congenic strains. By repeatedly backcrossing one strain onto another, it is possible to produce mice that have a particular genomic region from one strain and the remainder of their genome from the other. The effect of the introgressed genetic region derived from one strain (generally corresponding to a QTL) can then be specifically tested on the genetic background of the other strain. Liu et al. strengthened their results by generating congenic strains where the introgression of Klk loci on chromosome 7 from NZW strain onto the genetic background of B6 led to severe nephritis. Importantly, in a separate study by the same group, a gene transfer approach by systemic adenoviral delivery in congenic mice demonstrated the protective role of this gene in experimentally induced autoimmune glomerulonephritis [2]. Sequencing analysis of the five most differentially expressed Klk genes in mice identified polymorphisms in the promoter regions, and reporter assays on the Klkb3 promoter showed that genetic variants in this promoter affect gene expression. To translate their findings to humans, the authors performed three independent association studies and showed a genetic association of SNPs located within human Klkl gene and the Klkb3 promoter region with lupus and lupus nephritis.

The recent advances in genomic technologies allowing testing of elevated numbers of genetic variations, such as single nucleotide polymorphisms (SNPs), in cases and controls have revolutionized common disease genetics [3]. The genetic dissection of SLE has resulted in the identification of >20 genetic loci associated with host immune responses [4]. Lupus nephritis and anti-GBM disease share a common pathophysiology where intrinsic or planted kidney antigens are the driving force of mechanisms involving an interaction between primary immune responses and intrinsic kidney factors [5]. In addition to this complex genetic architecture, modest sample sizes obtained from both autoimmune disorders have been a major obstacle...
for gene identification by GWA because of insufficient statistical power to detect association. Given the increased heritability, flexibility and statistical power of experimental rodent crosses over the corresponding studies in humans, rodent models were previously successfully used in gene identification for immune-mediated glomerulonephritis [6,7]. The present work by Liu et al. uses genetical genomics, a combination of transcriptome analysis, genetic linkage and fine mapping, to identify genes in the mouse anti-GBM model. As a result, the Klk gene family was positionally cloned in mice, and the human translational studies confirmed the association with lupus nephritis. Although mouse chromosome 7 and human chromosome 19q13 were previously found to be associated with SLE [8–10], this is the first description of the role of the expression level of the Klk family in immune-mediated glomerulonephritis.

Tissue and plasma kallikreins are serine proteases that produce biologically active vasodilator kinin peptides from endogenous kininogen substrates, and the contribution of the kallikrein–kinin system is well established in the pathogenesis of hypertension and end-stage renal disease [11]. Since kallikrein expression was found to be in endothelial and mesangial cells [12,13], the renoprotective effect of kallikreins in the pathophysiology of glomerulonephritis could be through antifibrotic mechanisms (Figure 1). This is in agreement with a recent study showing that kallikrein gene knock-down by small interfering RNA transfection induces a profibrotic phenotype in rat mesangial cells [12]. Previous studies have shown that adenosiviral delivery of human kallikrein led to reduced hypertension and provided protection against renal injury in the spontaneously hypertensive and the streptozotocin-induced type 2 diabetic rat models [14,15]. More recently, the therapeutic effect of human kallikrein on chronic renal failure was also demonstrated in nephrectomized rats [16]. In the present study, the variation in Klk expression was established by microarray studies but the differential expression analysis was carried out on the mouse renal cortex, and further studies focusing on the precise tissue localization of kallikreins will facilitate the understanding of the mechanism(s) by which this gene family protects against glomerulonephritis. Congenic studies carried out by the same group showed that the Sle3 QTL on chromosome 7 controls T-cell activation, differentiation and cell death, and it could be hypothesized that Klk genes may have an impact on these pathways [8]. Bone marrow transplant experiments between congenic and parental B6 mice will be important in understanding the relative impact of intrinsic renal factors and circulating cells on glomerulonephritis.

Human Klk1 and the kallikrein-related peptidases are encoded by 15 genes localized in tandem on chromosome 19q13.4 and represent the largest cluster of protease genes in the human genome [17]. As pointed out by the authors, this region possesses many regulatory elements sensitive to steroid hormones extensively studied in prostate cancer. Polymorphisms and expression levels of Klk3, more commonly known as the prostate-specific antigen (PSA), could now be studied in autoimmune glomerulonephritis. Gene expression is a complex network under the control of cis- (the physical location of the gene itself) or trans- (genomic region that is distant from the physical location of the gene) regulatory control elements. It is noteworthy that a gene family rather than a single gene is underexpressed in NTN-susceptible strains, and the congenic, sequencing and reporter analyses suggest cis-regulation within the Klk locus; however, the mechanism(s) responsible for the overall Klk family underexpression are yet to be determined. This also underlines the importance of gene pathways rather than individual gene effects on polygenic traits, and recent views highlight the potential impact of genetic identification of biological pathways on translational research [18].

The recognition of the role of the Klk pathway in immune-mediated glomerulonephritis by nephrologists, and further basic research focusing on mechanisms by which Klk expression is renoprotective, should facilitate identification of novel drug targets for treatment of patients with glomerulonephritis.

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References
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