Molecular Genetics of Renal Diseases

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Recent progress in molecular genetics has strongly influenced nephrology. Gene mutations have been identified which cause various monogenic hereditary renal diseases including Alport syndrome, autosomal dominant polycystic kidney disease, and tubular transporter disorders. The data obtained will be useful not only to develop new methods of diagnosis and treatment of such particular diseases but also to expand the knowledge of renal physiology and pathophysiology. In addition, genetic factors have been investigated which are involved in the development and progression of more common renal diseases such as IgA nephropathy and diabetic nephropathy. There have also been great advances in molecular studies of experimental renal diseases such as Heymann nephritis and in the use of transgenic and knockout mice. In this review we focused on the important achievements made recently in the field of molecular nephrology.

(Key words: Alport syndrome, autosomal dominant polycystic kidney disease, tubular transporter disorders, IgA nephropathy, diabetic nephropathy, experimental renal diseases)

Introduction

Patients with renal failure due to various renal diseases have been increasing in number. Replacement treatments such as dialysis and renal transplantation are effective but costly and still far from compensating for native kidney functions. Therefore, it is one of the urgent medical issues to elucidate the molecular mechanisms of the renal diseases for preventing the deterioration of renal functions.

Recent advances in molecular genetics have made a great impact on nephrology. Gene mutations causing monogenic renal diseases (Table 1) have been identified by a method of so-called reverse genetics or by investigating candidate genes that had been speculated to be defective or localized in the genetic loci linked to the diseases. Furthermore, identification of numerous polymorphic gene markers has also made it possible to carry out genetical studies of putative polygenie renal diseases such as glomerulonephritis and diabetic nephropathy. In addition, molecular studies on animal renal disease models and the use of transgenic and knockout mice have become powerful tools to get insight into the pathogenesis of human renal diseases. In this review we focused on the recent progress in such molecular studies that identified or characterized specific gene products associated with renal diseases.

Alport Syndrome and Thin Basement Membrane Disease

Alport syndrome (AS) is a hereditary progressive nephropathy characterized by lamellation and splitting of the glomerular basement membrane (GBM) and is usually associated with sensorineural deafness and ocular defects. The disease occurs at a gene frequency of 1/5,000 and is transmitted in most families as an X-linked dominant trait (1). A variety of mutations in the COL4A5 gene which encodes the α5 chain of type IV collagen, a component of the GBM, have been identified in X-linked AS families (2). The mutations include single base substitutions, large deletions, and other rearrangements such as inversions, insertions, and duplications; but these are dispersed in the gene and no “hot spots” have thus far been identified. In Japanese patients with X-linked AS, only a case whom we reported was affected by a large deletion (3) but most of the others were due to single base mutations, and small size deletions and insertions (4). Female patients with X-linked AS usually lead a milder clinical course than males, but females with severe phenotypes were found to result from preponderant inactivation of the X chromosome carrying the normal allele (5).

The COL4A3 and COL4A4 genes that encode the α3 and α4 chains of type IV collagen, respectively, were identified and colocalized in a head-to-head fashion on human chromosome 2 (6). Because these chains are also contained in the GBM, mutations in the genes were screened for AS families in which consanguinity suggested autosomal recessive inheritance. Homozygous mutations were found in both genes (7), indicating that there is an autosomal form of AS in addition to the X-linked form.

The association of AS with diffuse esophageal
Table 1. Major Monogenic Renal Diseases and the Genes Mutated

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Genes</th>
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<tbody>
<tr>
<td>Alport syndrome (X-linked)</td>
<td>type IV collagen α5 chain (COL4A5)</td>
</tr>
<tr>
<td>Alport syndrome (autosomal recessive)</td>
<td>type IV collagen α3 chain (COL4A3) or α4 chain (COL4A4)</td>
</tr>
<tr>
<td>Alport syndrome with leiomyomatosis (X-linked)</td>
<td>type IV collagen α5 and α6 chains (COL4A5 and COL4A6)</td>
</tr>
<tr>
<td>benign familial hematuria (a Dutch family, autosomal dominant)</td>
<td>type IV collagen α4 chain (COL4A4)</td>
</tr>
<tr>
<td>autosomal dominant polycystic kidney disease 1</td>
<td>polycystin (PKD1)</td>
</tr>
<tr>
<td>autosomal dominant polycystic kidney disease 2</td>
<td>(PKD2)</td>
</tr>
<tr>
<td>nephrogenic diabetes insipidus 1 (X-linked)</td>
<td>vasopressin V2 receptor (ADHVR2)</td>
</tr>
<tr>
<td>nephrogenic diabetes insipidus 2 (autosomal recessive)</td>
<td>aquaporin 2 (AQP2)</td>
</tr>
<tr>
<td>Liddle’s syndrome*</td>
<td>amiloride-sensitive epithelial Na⁺ channel</td>
</tr>
<tr>
<td>pseudohypoaldosteronism type 1</td>
<td>amiloride-sensitive epithelial Na⁺ channel</td>
</tr>
<tr>
<td>Gitelman’s syndrome</td>
<td>thiazide-sensitive Na-CI cotransporter</td>
</tr>
<tr>
<td>familial hypocalciuric hypercalcemia</td>
<td>Ca²⁺ sensing receptor</td>
</tr>
<tr>
<td>neonatal severe hyperparathyroidism</td>
<td>Ca²⁺ sensing receptor</td>
</tr>
<tr>
<td>autosomal dominant hypocalcemia*</td>
<td>Ca²⁺ sensing receptor</td>
</tr>
<tr>
<td>Dent’s disease</td>
<td>Cl⁻ channel (CLCN5)</td>
</tr>
<tr>
<td>X-linked recessive nephrolithiasis</td>
<td>Cl⁻ channel (CLCN5)</td>
</tr>
<tr>
<td>X-linked recessive hypophosphataemic rickets</td>
<td></td>
</tr>
</tbody>
</table>

*Caused by activating gene mutations.

leiomyomatosis (DL) has been described (8). The DL-AS patients were found to be affected by gene deletions that involve the 5' ends of COL4A5 as well as COL4A6 that encodes a novel α6(IV) collagen chain and is closely located at the upstream of COL4A5 (9). Recently DL-AS was implied to be caused by expression of an abnormal truncated α6(IV) chain rather than its defect by a large deletion of the gene (10). The α1(IV) and α2(IV) chains are found ubiquitously in basement membranes (BMs) but the α3(IV), α4(IV) and α5(IV) chains have restricted tissue distributions (11). However, the α6(IV) chain is absent in the GBM although it is colocalized with the α5(IV) chain in many BMs (12). This indicates that the α5(IV) and α6(IV) chains are not always coordinately regulated and excludes COL4A6 as a gene mutated in AS. The α3(IV), α4(IV), α5(IV) and α6(IV) chains were found to be absent from all renal BMs in patients with X-linked AS while the α1(IV) and α2(IV) chains were increased (12). The data supports the existence of two independent collagen networks, one for the α1(IV) and α2(IV) chains and the other for the α3(IV), α4(IV), α5(IV) and α6(IV) chains. In the GBM, however, the α3(IV), α4(IV) and α5(IV) chains appear to form a particular network. Coordinate gene expression of the α3(IV), α4(IV) and α5(IV) chains in the kidney was also supported by an analysis using a canine model of X-linked nephritis with a COL4A5 gene mutation (13).

Patients with thin basement membrane disease (TBMD) or thin glomerular basement membrane disease exhibit persistent microscopic hematuria with a diffuse thinning of the GBM, especially of the lamina densa. The patients rarely lead to end-stage renal disease (ESRD) in contrast to AS. Familial TBMD, also known as benign familial hematuria, is inherited in an autosomal dominant mode (1). The disease might be a variant of AS because AS often shows thinning of the GBM at its early stage (14). It was reported that COL4A1 and COL4A2 are not linked to TBMD (15). In addition the parents of patients with autosomal recessive AS caused by mutations in the coding region of COL4A3 or COL4A4 should be heterozygous for the mutations but do not usually show abnormal urinalysis data (7). We have investigated a possibility that in TBMD there may be genetic abnormality in the regulatory regions of COL4A3 or COL4A4 which might result in decreased expression of the GBM components, but the study identified no linkage to the COL4A3 locus (as well as COL4A4 adjacent to COL4A3) in the four Japanese families (16). A recent analysis, however, revealed that benign familial hematuria in a Dutch family was caused by a single-base COL4A4 mutation (17), suggesting that the disease may result from heterogeneous causes.

Although the primary genomic defects were identified that directly affected the structure of the GBM in AS, it remains unknown why this leads the patients (especially males) to ESRD. Also, the reason why TBMD patients do not fall into ESRD is not clear. Answers to the questions may be helpful to elucidate the molecular mechanisms of development of ESRD which might be associated with a putative common pathway of progression of various renal diseases.

Autosomal Dominant Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common human monogenic disorders, affecting approximately 1 in 1,000 individuals, and accounts for ~8% of ESRD (18). The disease is characterized by the
progressive formation and enlargement of renal cysts, resulting in the destruction of the normal kidney architecture. Other manifestations of the disease include hepatic cysts, cardiac valve abnormalities, and cranial aneurysms, indicating that ADPKD is a systemic disorder.

Genetic heterogeneity in ADPKD has been demonstrated after linkage was first confirmed for the gene on chromosome 16p13.3 (PKD1) (19) which accounts for ~85% of ADPKD families. The second gene, PKD2, has been localized on chromosome 4 (20, 21) and accounts for approximately 15% of ADPKD families. In addition, a third gene, PKD3, has been suggested to exist (22, 23). Non-PKD1 families exhibit an identical spectrum of organ involvement but slower development of ESRD than PKD1 families.

After long pursuit by positional cloning, the PKD1 gene was identified by analyzing a chromosomal translocation in a family with PKD1 accompanied by tuberous sclerosis that had been found to result from a defect of a gene located near PKD1 (24). Characterization of the breakpoint in 16p13.3 led to the identification of PKD1 in which mutations were found in PKD1 families (24). Cloning of the entire gene (25, 26) and the full-length cDNA (26, 27) made it possible to predict that the PKD1 product, designated polycystin, consists of 4,303 (26) or 4,302 amino acids (27) that code for a putative transmembrane protein with novel repeat motifs in its extracellular domain.

Widespread expression of PKD1 mRNA was seen in human adult tissues, with high levels in the brain and moderate in the kidney (28). Expression of the PKD1 protein, polycystin, was localized to the tubular epithelium (28, 29) and glomerular parietal and visceral epithelium (podocytes) (29) in fetal and adult kidney, suggesting that polycystin functions for the maintenance of renal epithelial differentiation and organization (28). In addition, polycystin mRNA and protein expression appears higher in cystic epithelia, indicating that PKD1 does not result from complete loss of the protein (28, 29).

Recently the PKD2 gene was also identified by positional cloning (30). Expression of the mRNA was shown to be ubiquitous in various tissues. The predicted 968-amino acid sequence of the PKD2 protein has six transmembrane spans with intracellular N- and C-termini. The region containing the six transmembrane segments but the N- and C-terminal domains has amino acid similarity with the PKD1 protein region that contains its four predicted transmembrane segments. However, the PKD2 protein appears more likely to belong to the family of voltage-activated calcium and sodium channels, suggesting that the PKD2 protein could also function as an ion channel or pore. Given that the clinical courses of PKD1 and PKD2 are very similar, it is speculated that the PKD1 and PKD2 proteins are involved in a common signal transduction pathway.

Hereditary Disorders of Tubular Transporters

Nephrogenic diabetes insipidus is characterized by insensitivity of the renal concentrating system to the water-sparing effects of the antidiuretic hormone arginine vasopressin (ADH). The congenital forms include X-linked recessive and autosomal recessive types. The former type has been found to result from various mutations in a gene (ADHRV2) that encodes the V2 ADH receptor in the collecting tubular cells since the first report of the mutation (31). A male patient of the latter type was also demonstrated to be affected by heterozygous gene mutations (32) of aquaporin-2, a water channel in the collecting tubule (33).

Liddle’s syndrome is a rare autosomal dominant form of hypertension characterized by hypokalemia and decreased serum renin activity despite of suppressed secretion of aldosterone. The disease has been speculated to result from increased activity of an amiloride-sensitive epithelial Na⁺ channel in the cortical collecting tubule which might allow sustained absorption of Na⁺ and result in extracellular volume expansion. Recently three subunits (α, β, and γ) of the epithelial Na⁺ channel were cloned (34, 35) and gene mutations in the β and γ subunits were actually found to cause Liddle’s syndrome (36, 37). The activating gene mutations result in the increase in number of Na⁺ channels at the apical membrane, which would increase renal Na⁺ absorption and create a predisposition to hypertension (38). In contrast, mutations in the subunits of the Na⁺ channel which result in the loss of the activity were found in patients with autosomal recessive pseudohypoaldosteronism type I (39), a rare salt wasting disease which is characterized by dehydration, hyponatremia, hyperkalemia and metabolic acidosis often in the neonatal period, and actually appears as a clinical mirror image of Liddle’s syndrome.

Gitelman’s syndrome, a variant of Bartter’s syndrome, refers to a predominant subset of patients with hypokalemic alkalosis associated with hypocalciuria and hypomagnesemia. The thiazide-sensitive Na-Cl cotransporter of the distal convoluted tubule was cloned (40) and the gene mutation was shown to cause Gitelman’s syndrome (41). Familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism were found to be caused by inactivating gene mutations of a recently cloned extracellular Ca²⁺-sensing receptor that is expressed in the parathyroid and kidney (42). By contrast, an activating mutation in the gene causes autosomal dominant hypocalcemia (42). Three hereditary disorders of hypercalciuric nephrolithiasis (Dent’s disease, X-linked recessive nephrolithiasis, and X-linked recessive hypophosphataemic rickets) were found to be caused by mutations of the same gene, CLCN5, which encodes a renal Cl⁻ channel (43). These disorders show low-molecular-weight proteinuria and hypercalciuria, suggesting the presence of tubulopathy. It remains to be investigated how the Cl⁻ channel impairment is associated with the tubular dysfunction.

These hereditary tubular diseases are rare but the identification of the disease-causing genes will contribute to understanding the functions of the gene products and exploring the pathogenesis of other diseases that are more common. For instance, an impaired regulation of the epithelial Na⁺ channel may participate in the pathogenesis of essential hypertension.
Genetic Studies on Putative Polygenic Renal Diseases Such as Glomerulonephritis and Diabetic nephropathy

Common renal diseases such as glomerulonephritis and diabetic nephropathy have also recently become subject to genetic studies. These diseases are often developed sporadically but some genetic factors are implicated to be involved (44-47). The putative genetic factors may be divided into two categories: one is for factors involved in initiating the particular diseases, and the other for those associated with progressing ESRD which may be inherited independently of the former ones. As candidates for the first category of factors, HLA allotypes and haplotypes have been intensively studied in patients with glomerulonephritis (45, 46), although the pathogenetical roles have not been completely determined. The second category of factors, which may be associated with the occurrence of ESRD regardless of the basic diseases, has been suggested in racial and familial studies (47). Out of these, angiotensin-converting enzyme (ACE) gene polymorphisms have been most studied.

An insertion (I)-deletion (D) polymorphism in intron 16 of the ACE gene is associated with variation in circulating levels of ACE: The DD genotype shows the highest ACE level, followed by the DI, then II genotypes (48). The D allele is associated with various conditions including myocardial infarction (49) and cardiomyopathy (50). The D allele was reported to be associated not with development of IgA nephropathy but with rapid progression of renal failure in the patients (51-53). In addition, IgA nephropathy patients with the DD genotype were shown to respond to ACE inhibition therapy with lisinopril for decreasing proteinuria (52), but there is a discordant report showing that enalapril did not lower the degree of proteinuria in non-diabetic renal disease patients with the DD genotype (54). The presence of nephropathy in insulin-dependent diabetes mellitus (IDDM) was reported to be associated with the D allele (55). However, Schmidt et al indicated that there was no such association in nephropathy both due to IDDM and non-insulin-dependent diabetes mellitus (NIDDM) (56). On the other hand, there is a report indicating that the DD genotype appears to be associated with higher urinary albumin excretion in patients with nephropathy in NIDDM patients (57). In addition, a recent report by Yoshida et al demonstrated that the DD genotype has a significant prognostic value for progression of nephropathy in NIDDM (58). These results suggest that, as in the case of IgA nephropathy, the ACE gene polymorphism appears to be associated with progression of diabetic nephropathy.

These association studies should be confirmed by investigating more patients whose clinical features are correctly characterized. Furthermore, affected sib-pair studies using multiplex families may need to be carried out to study the genetic factors as done for other putative polygenic disorders such as IDDM (59) and Crohn’s disease (60).

Progress in the Molecular Study of Experimental Nephrology

Heymann nephritis (HN) is a well-characterized experimental autoimmune model of membranous nephropathy, one of the most common primary diseases that cause nephrotic syndrome in adults. As a major autoimmune target in HN, gp330 was identified which is located in the clathrin-coated pits of glomerular podocytes and proximal tubular cells. We have elucidated the primary structure of rat gp330 by cDNA cloning (61). gp330, which we renamed megalin, consists of 4,660 amino acids and has a predicted molecular weight of 517 kDa without glycosylation. It functions as an endocytic receptor of multiple ligands and is involved in Ca\(^{2+}\) sensing (62). Using recombinant megalin fragments we mapped its pathogenic epitope involved in the formation of immune deposits in HN (63), which would promote the analysis of the molecular mechanisms of HN.

Transgenic and gene-targeting technology has become a powerful tool for experimental nephrology (64). First, it has provided information about novel proteins associated with renal diseases: A transgenic mouse strain which showed nephrotic syndrome and glomerulosclerosis was developed by a genomic insertion of a single provirus (65). The gene inactivated by the insertion, referred to as Mpvl7, was found to encode a peroxisomal protein producing reactive oxygen species (ROS), suggesting a link between peroxisomal ROS production and glomerulosclerosis (66). Second, gene targeting has revealed developmental or physiological functions of targeted gene’s products in the kidney: Mice deficient for platelet-derived growth factor (PDGF)-B or PDGF \(\beta\) receptor showed no formation of glomerular tufts apparently because of absence of mesangial cells, indicating their role in migration of mesangial cells into glomeruli (67). Third, it has developed new renal disease models: Mutant mice lacking laminin \(\beta_2\), a normal GBM component, revealed albuminuria despite of no apparent renal structural abnormality except for fused foot processes of podocytes, resembling minimal change nephrotic syndrome in humans (68).

Experimental gene transfer into the kidney has been conducted in an attempt to unravel the mechanisms of renal diseases and develop novel methods of the treatment (69). Gene therapy of experimental fibrotic glomerulonephritis was also shown to succeed by skeletal muscle gene expression of decorin, an inhibitor of transforming growth factor-\(\beta\)1 (70). There are still many technical and ethical problems to be resolved for applying gene transfer \textit{in vivo} to treatment of human diseases, but it certainly has the potential to become an effective therapeutic tool in the future.

Conclusions and Perspectives

Identification of disease-causing or disease-associated genes has strongly influenced the development of clinical methods of diagnosis and treatment of specific renal diseases. It has also expanded our knowledge on renal physiology and pathophys-
logy by analyzing the phenotypes caused by the defective gene products. The knowledge will again give clinical studies feedback on elucidating the pathogenesis of more complex renal diseases. Availability of a large number of polymorphic makers in the whole human genome also will be a powerful tool for analyzing complex genetic factors which may be involved in various forms of glomerulonephropathy. The analysis of experimental renal disease models and the continued use of transgenic and knockout mice are expected to provide more insight into human disease counterparts. In addition, new techniques developed (71) may promote isolating more genes that are predominantly expressed in the kidney. These genes may be found to regulate critical functions of the kidney in homeostasis of water and electrolytes, metabolism of numerous molecules and synthesis of hormones and, in turn, cause certain renal diseases when mutated.

Barter’s syndrome, featuring salt wasting, hypokalemic alkalosis, hypercalciuria and low blood pressure, was found to be caused by mutations in the Na-K-2Cl cotransporter (NKCC2), a mediator of salt reabsorption in the thick ascending limb of the loop of Henle (72). In the kindreds that show no linkage to the gene, mutations was identified in an ATP-sensitive K+ channel (ROMK) that recycles reabsorbed K+ back to the tubular lumen (73). These findings indicate the genetic heterogeneity of Barter’s syndrome and support the physiological role of ROMK in the regulation of the renal NKCC2 activity.

References


