18 Genomic Methods in the Diagnosis and Treatment of Pediatric Kidney Disease

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The completion of the Human Genome Project (HGP) in 2003 has laid the foundation and driven the technological advancements necessary for the study of the genetics of complex, multi-factorial diseases, such as those affecting the kidney. The International HapMap Project has built upon the HGP through the systematic identification and cataloguing of genetic variation across human populations. Translating the mass of data generated by these studies into useful clinical knowledge is now a major undertaking in nearly all areas of medicine, including the field of Pediatric Nephrology. Much of this work will revolve around linking particular patient phenotypes to genomic and proteomic data, such as genotype, expression profile, and protein biomarkers. As evidenced by the etiological advances made in various kidney disorders resulting from the application of genome-wide linkage analyses in the 1990's, there are a number of unique aspects of Pediatric Nephrology that make it an area particularly suitable to genomic exploration with such novel technologies as genome-wide association and expression analyses. These aspects relate both to the clinical characteristics, as well as to public health and epidemiological concerns, of pediatric kidney diseases.

A number of pediatric kidney diseases are considered to have a multi-factorial pathogenesis, often reflected in their variable clinical presentations. Such diseases are often difficult to diagnosis; and invasive biopsies are often required. Genotype and/or expression information obtained from large-scale association or profiling studies may refine the diagnosis of a number of kidney diseases and even allow for individualized disease management and therapy. Additionally, there remains an urgent need for clinically useful biomarkers which can assist in the prevention and early diagnosis of both acute and chronic kidney diseases in children. Novel serum and/or urine biomarkers identified through the application of high-throughput proteomics offers another possibility for enhanced diagnosis and treatment. Moreover, from a public health standpoint, proper management of renal disease is expensive, particularly renal replacement and dialysis. The treatment of kidney patients based on genomic and proteomic data may help lessen this burden by enhancing disease risk prediction and creating disease subsets with associated prognostic and therapeutic implications. While these issues are relevant to all of nephrology in general, as well as to many other areas of medicine, they are particularly germane to the study of childhood kidney diseases where there can be high mortality, the need for expensive lifetime treatment, as well as serious growth and nutrition implications. The proper application of genomics in Pediatric Nephrology is well-suited to address the unique challenges posed by this field.

This chapter aims to provide an overview of the application of genomic technologies to the study of pediatric kidney diseases. We will begin with a discussion of the polygenic nature of complex diseases and the recent genomic advances enabling their study. This will include outcomes of the HGP and International HapMap Project. We will then discuss the application of various genomic methods to the more common pediatric kidney diseases, covering the range of both chronic and acute, with a focus on the DNA level. We will end with a discussion of the future prospects and recommendations for applying genomics to pediatric nephrology.

The Genomics Revolution

In 2003, the completion of the Human Genome Project brought the scientific community one step closer to identifying the genes underlying common, polygenic diseases. Prior to this achievement, the goal of identifying the genetic factors responsible for diseases presenting substantial public health burdens was elusive. Research efforts focused on monogenic disorders following Mendelian patterns of inheritance. Linkage analysis was the main tool for the identification of genes behind these monogenic diseases. Although linkage analysis proved to be an effective method for the mapping of numerous genes in monogenic disorders during the 1980's and 90's, including for familiar forms of various kidney diseases (1–5), it's subsequent application to complex diseases did not meet

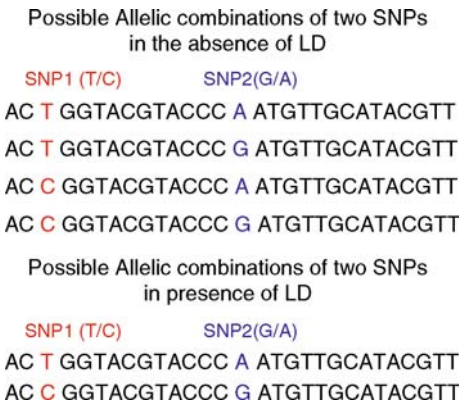
with the same success. As linkage analysis is more suitable for the identification of genes with relatively large effect sizes, its application to polygenic diseases, where the contribution of each locus or allele is believed to be relatively small, resulted in less than significant findings and results that could not be replicated. Only a handful of genes contributing to common diseases have been identified to date through linkage and subsequent positional cloning methods (6–9).

In 1996, Risch and Merikangas demonstrated the power of genome-wide association analysis (GWA) over linkage methods for the identification of the genes behind complex diseases (10). Association analysis relied upon the availability of a standardized dense set of markers covering the genome that could be easily and inexpensively typed, as well as sample sizes of thousands of cases and controls. Until the technology existed to identify, accurately map and then genotype such large numbers of markers in such great numbers of individuals, linkage analysis followed by fine-mapping remained the best option at that time for uncovering complex disease genes. However, the work of the HGP began to drive the technological breakthroughs needed to map polygenic diseases.

Analysis of the human genome sequence has demonstrated the abundance and uniformity of single nucleotide polymorphisms (SNPs), single base changes in DNA that occur in at least 1% of the population (This is in contrast to a mutation, which occurs in < 1% of the population). It is some of these common DNA variants that are believed to be responsible for at least some of the phenotypic variation observed within and between populations, including various diseases. The HGP contributed to developing technologies for the rapid, large-scale identification and scoring of SNPs and creating the intellectual resources needed to study sequence variation. The International HapMap Project built upon these discovery efforts by characterizing the patterns of linkage disequilibrium (LD) and haplotype structure across the human genome. LD occurs when two or more markers segregate together with significantly different frequencies than would be expected if they segregate independently from each other. LD is generally greater for SNPs in close physical proximity. As LD tends to reduce the number of possible haplotypes, or set of alleles, present in a population, it is useful for association mapping, in that knowing the genotype of one marker can predict the genotype of another marker in LD with it (*Fig. 18-1*). The work of the HapMap Project, along with others, has resulted in the identification, mapping, characterization, and the public availability of over four million validated SNPs to date (www.hapmap.org). The identification, mapping and cataloging of these SNPs in various human populations has helped make association studies a reality. In conjuction with the advent of mass throughput genotyping platforms, such data has potential to contribute extensively to our basic understanding of human biology and disease.

Figure 18-1

The Prinicipal of Linkage Disequilibrium. A Set of DNA sequences illustrating two SNPs. In the top half, the SNPs are not in LD with each other and segregate independently. With two SNPs in linkage equilibrium, four possible allelic combinations are possible. In the bottom half, two SNPs in the presence of LD are illustrated. Notice that LD reduces the number of possible allelic combinations, as the SNPs no longer segregate independently. Here, the 'T' allele of SNP1 segregates only with the 'A' allele of SNP2, while the 'C' allele of SNP1 segregates only with the 'G' allele of SNP2. In such a case, only one of these two SNPs would need to be genotyped, as the genotype at one SNP will provide the genotype information for the other.



The two types of association studies that are commonly used are the candidate-gene approach and the genome-wide approach (*Table 18-1*, *Fig. 18-2*). Candidate-gene association studies rely upon a specific hypothesis and are often limited by our current knowledge of biological and/or disease mechanisms. The strategy has been a frequently employed method in the study of numerous traits and diseases, including many related to nephrology. Nevertheless, replicable results have been difficult to achieve for various reasons, including the small, underpowered sample sizes characteristic of many of these types of studies (11).

In the second type of association study, the genome-wide strategy is hypothesis-free and does not make any assumptions regarding the mechanisms behind the phenotype being studied. Genome-wide association studies are ideally suited for common complex diseases, where

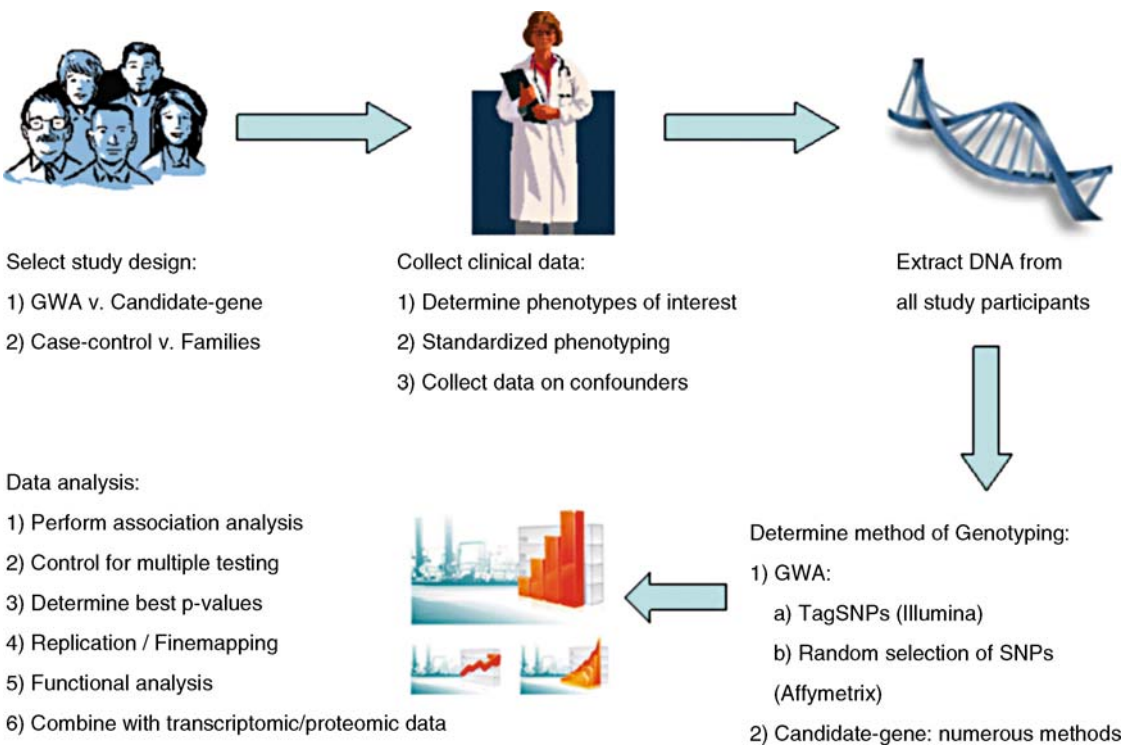
Table 18-1

Comparison of Genome-wide (GWA) and Candidate-gene Association Studies

Study design	Advantages	Disadvantages
GWA	Hypothesis-free	Genotyping more technically demanding
	May be more economical and efficient when large numbers of loci need to be worked-up	May be costly and time-consuming
	Ideal in cases where little is known regarding disease mechanisms	
	Often allow for collection of data relating to copy number polymorphisms (CNPs) in addition to SNPs – two genetic variants for the price of one.	Requires sophisticated bioinformatic approaches to data analysis and storage
Candidate-gene	Less costly and time-consuming if large numbers of loci do not need to be analyzed	Candidate-gene selection constrained by known disease biology
	Ideal in situations where evidence implicates particular disease mechanisms and related genes; or when following-up a QTL containing likely functional candidates	Less economical and efficient when testing great numbers of loci
	Straightforward data-handling and analytical approaches	
	Various methods of genotyping available; less technically demanding	

Figure 18-2

Workflow of a Genetic Association Study.



the physiological mechanisms and genetic factors that contribute to them are often not well understood. GWA studies are based on hundreds of thousands to millions of SNPs spread across the genome, each one tested for association with a particular outcome. There are currently two main approaches to this type of study. One type employs an LD-based selection strategy, as exemplified by the Illumina HumanHap-550 and Human1M sets of variants. The LD-based strategy exploits patterns of genomic LD to select a minimum number of SNPs (tagSNPs) to be assayed which captures the maximum amount of information across the genome. SNPs on these arrays serve as proxies for those not assayed. The second approach ignores patterns of LD and relies on the random selection of markers. This approach is the basis for the Affymetrix 500 K and 1 million SNP array products. A number of GWA reports have recently been published identifying novel genes for various diseases in adult populations, including many outcomes relevant to kidney disease, such as obesity and Type II Diabetes (12–14), as well as quantitative traits like urinary albumin excretion (15).

Within the field of Pediatric Nephrology, many of the known disease genes were initially identified through the study of familiar forms of a disease using linkage analysis followed by positional cloning methods (16–19). These genes have become obvious candidates to examine in the context of seemingly sporadic occurrence, where a patient has no prior family history of the disease, and which constitutes the majority of many childhood-onset kidney diseases. This is often done through mutation scanning in case series. While linkage analysis proved to be a useful tool for identifying the first disease genes in pediatric kidney disease, allowing for some of the first insights into the molecular pathogenesis of many of these diseases, these genes generally only account for a minority of cases. New methods which result in the identification of novel genes can assist in making further advances. While GWA studies are still relatively new, candidate gene studies have been an actively pursued approach in nephrology for novel gene identification. The popularity of this approach is illustrated by a review of the 2004 and 2005 abstracts of the American Society of Nephrology (ASN) and the European Renal Association/European Dialysis and Transplantation Association (ERA/EDTA), where over 180 studies encompassing 205 polymorphisms in 92 different genes were submitted (20). The genes most frequently studied were those of the renin-angiotensin system (RAS) and those related to inflammation (20). (The best evidence to date indicates minor statistical associations and a negligible clinical relevance of the most frequently studied RAS polymorphism, the insertion/deletion (I/D) variant of the angiotensin I-converting enzyme (ACE) gene (21–23). Despite their frequent study, a genetic association has yet to be convincingly demonstrated for any of the RAS genes. These genes will not be further discussed in the chapter. Of particular note, the authors highlight the extremely small sample sizes of the vast majority of these studies. In 2004, the average study size was 185 (cases and controls combined), with a slight increase in 2005 to 276. In addition, the authors found that while only 16% of these studies met the newly-adopted criteria for publishing candidate gene association studies in the Journal of the American Society of Nephrology (JASN) in 2004, this number jumped to nearly half in 2005 (20). These data highlight two important points. First, as it is unlikely that the often thousands of subjects needed for adequately-powered studies can be collected at a single site, there is a need for large-scale, multi-site collaborative studies. Large-scale candidate-gene association studies of novel genes in adult or pediatric populations are currently lacking. Second, by adopting strict publishing guidelines, journals can help assure the proper design and analysis of association studies. To address the first point, European investigators interested in nephrology and genetics established the Renal Genome Network (ReGeNet; www.regenet.eu) in 2003 to encourage large, collaborative clinical studies focused on the genetic epidemiology of renal disease (24). To date, a comparable formal renal network of investigators is lacking in the States. Such a network dedicated to studying the genetic basis of pediatric renal disease would greatly assist in establishing the infrastructure needed to design and implement adequately-powered association studies, whether those studies are candidate-gene-based or genome-wide.

When many genes are to be tested, or when one desires a hypothesis-free approach, it may be more efficient and economical to perform a GWA study. While these studies may assist in the identification of novel genes in acute kidney disorders, where currently half or less of all cases are explained by known mutations, GWA approaches in pediatric nephrology are likely to be most beneficial when applied to chronic kidney disease (CKD) and end-stage renal disease (ESRD) resulting from the increasing rate of hypertension, diabetes and obesity in children. The mechanisms leading to CKD and ESRD in these settings are likely to be polygenic in nature and the current lack of understanding regarding all disease mechanisms leading to these outcomes precludes a selection of appropriate candidate genes. In addition, the mechanisms of these disorders and their precipitating causes may be different from those occurring in adults. While some of the GWA findings from adult populations

may be relevant to pediatric populations as well, more work will be required to determine this. A more in-depth discussion on the application of candidate gene association and GWA approaches to the more common pediatric kidney diseases will be presented in the following section.

While much of this discussion has focused exclusively on the role of DNA sequence variation in disease outcomes, there are also extensive research efforts dedicated to the role of mRNA and proteins in the prevention, diagnosis and treatment of kidney disease. Expression profiling, as with high-throughput SNP genotyping, has benefited substantially from recent advances in microarray technology, which are allowing for the measurement of genome-wide expression levels, also called 'transcriptomics,' and their correlation with various disease states. As with cancer, the iden- tification of certain common expression 'signatures' through the simultaneous profiling of thousands of genes from kidney biopsy samples may help determine treatment course and patient prognosis in various renal diseases. The genome-wide study of proteins, or proteomics, has also benefited from recent technological breakthroughs in mass spectrometry, allowing for a greater number of proteins to be simultaneously analyzed. There is currently an urgent need in pediatric nephrology for clinically-relevant biomarkers which can be obtained non-invasively and which will eventually allow for earlier disease detection (25, 26). Because these two fields are closely aligned with the study of DNA sequence variation, in that changes affecting gene sequence can ultimately affect gene expression and protein production, we focus in the following section on the application of the study of DNA sequence variation to the various forms of pediatric renal disease. The application of all three fields to the study of pediatric nephrology can ultimately lead to enhanced prevention efforts, diagnoses and treatment options through the identification of novel genes and genetic markers, gene expression signatures and biomarkers associated with various renal disease states and outcomes in pediatric populations.

The Application of Genomic Methods to Pediatric Kidney Diseases

Acute Diseases

Table 18-2 provides a summary of the genes and loci implicated in the following conditions, as well as information on known genotype-phenotype correlations.

Nephrotic Syndrome (NS) NS as a heterogeneous collection of disorders represents an excellent example of where the application of genetic and genomic methods can assist in a greater understanding of disease pathogenesis and treatment.

In children, minimal change nephropathy (MCN) is the overwhelming cause of NS, such that renal biopsies are often not performed before beginning treatment with potentially harmful immunosuppressive therapies (27). Corticosteroids are often prescribed first. The majority of children respond well to this treatment and they are said to have 'steroid-sensitive nephrotic syndrome' (SSNS). However, there is a subset of children who do not respond to this therapy and they are said to have 'steroid-resistant nephrotic syndrome' (SRNS). In such cases, a renal biopsy may be indicated and the underlying cause may be focal segmental glomerularsclerosis (FSGS). Current treatment strategies for MCN and FSGS are similar, however, they are generally less effective for FSGS; and children with the latter may go on to develop ESRD. Despite the existence of these therapeutic strategies, the underlying pathological mechanisms of these two histological classifications are poorly understood. It is a subject of debate in the literature as to whether or not these classifications represent distinct diseases or subtypes of a single condition (27). In addition to a need for a greater understanding of the disease processes involved in NS, less toxic therapies are also needed for treatment in children. In the last decade, much progress has been made in the area of NS genetics and more can be expected in the coming years with novel genomic technologies. These advances should help to clarify NS-related pathologies and offer a pharmacogenetic approach to the treatment of NS.

In the late 90's, genome-wide linkage analysis followed by positional cloning was a particularly successful method for the identification of the genes behind the familial form of NS, although sporadic cases, those without any previous family history, account for the majority of pediatric NS cases. In 1998, Kestila et al. identified the first NS-related gene, NPHS1 (nephrin), to be the cause of an autosomal-recessive form of congenital NS in the Finnish population (16, 17). This was a particularly severe form of NS, characterized by heavy proteinuria in utero, lesions of the glomerular filtration barrier, and often death within the first two years of life (17). These findings were quickly followed by the identification of the NPHS2 (podocin) gene and its mutations as the cause of autosomal recessive steroid-resistant NS in older children from families of Northern European and North African descent (18, 19). This form presented between three months and five years of age and was characterized by minimal glomerular changes on early biopsy samples, FSGS present at later stages and rapid progression to ESRD. Both nephrin and

Table 18-2

Genes and genotype-phenotype correlations in Selected Pediatric Renal Diseases

Disease	Gene(s)/Loci	Genotype-Phenotype Correlations	Comments
Nephrotic Syndrome (steroid-resistant)	NPHS1, NPHS2, LAMB2, WT1, PLCE1	NPHS1,NPHS2,LAMB2,WT1 commonly mutated in congenital onset & resistant to steroid treatment; Type & number of NPHS2 mutations correlates with age of onset.	Additional susceptibility genes and modifier genes likely. GWA studies may assist with their discovery.
Atypical Hemolytic Uremic Syndrome	CFH, CFI, CFB, MCP (complement regulatory genes)	CFH -severe clinical course, rapid progression to ESRD, earlier age at onset, disease recurrence after transplant; MCP -most favorable course, no recurrence post transplant; CFI – intermediate course.	Known complement genes account for ~50% of cases. Additional susceptibility genes likely. Modifier genes likely due to high incomplete penetrance. SNPs may also play a role along with known mutations.
Autosomal Dominant Polycystic Kidney Disease	PKD1, PKD2	PKD1 – more severe course & earlier onset; 5′ mutations may progress to ESRD earlier & be more susceptible to ICAs.	PKD1 accounts for ~85% of families. Most mutations in these genes are unique to single family. Genetic background may account for variability w/in a family. Medical re-sequencing can assist in increasing the numbers of particular mutational profiles. GWA studies may help to uncover modifier genes.
Autosomal Recessive Polycystic Kidney Disease	PKHD1	Presence of two truncating mutations results in neonatal death, regardless of position. Missense mutations associated with milder phenotype.	~33% of mutations are unique to a single family. Medical re-sequencing can assist in increasing the numbers of particular mutational profiles.
IgA Nephropathy	6q22–23; 4q26–31; 17q12–22; 2q36; megsin gene, uteroglobin, C1GALT1, E & L selectins, PIGR, IGHMBP2, TNFa, TGFb, IL-4, IFg, and HLA-DRA	Gene verification and discovery and genotype-phenotype correlations still needed.	No gene(s) yet identified in families. Many results from case-control studies need verification. GWA studies needed.
End-Stage Renal Disease	10p; 18q; See Iyengar, et al. for additional loci; D10S558, D10S1435, D6S281, D4S2937, D2S291, D17S515, CNDP1, ELMO1, PVT1	Gene verification and discovery and genotype-phenotype correlations in pediatric cohorts still needed.	Genes identified in adult populations and many in setting of DN. Verification in adults and children required. GWA studies needed.

podocin localize to the slit diaphragm and are key glomerular filtration barrier proteins.

Since the identification of these genes, numerous studies have investigated their role in all forms of NS. It is now well-established that 10–30% of sporadic NS cases with FSGS are due to mutations within NPHS2 (28). Mutations within the laminin-β-2 (LAMB2), Wilms' tumour 1 (WT1) and phospolipase C-ε (PLCE1) genes have also been reported in pediatric NS, although they are far less frequent (29–31). Hinkes et al. reported that in 89 patients from 80 families manifesting NS within the first year of life, mutations in one of four genes (NPHS1, NPHS2, WT1, and LAMB2) were identified in 66% of the cases, with nearly 38% and 23% due to NPHS2 and

NPHS1 mutations, respectively (32). When examined by age of onset, the proportion of congenital cases explained by mutations in one of the four genes was nearly twice that of infantile-onset cases. Also of note, the study reported that children with mutations in any one of the four genes did not respond to steroid treatment. The authors concluded that children with onset of NS during the first year of life should be screened for mutations within these four genes in order to guide therapeutic course; and that there are likely additional unknown genes playing a role in early-onset NS.

Previous studies have suggested both allelic and locus heterogeneity of NS. While nephrin mutations have been found only in patients with congenital onset, podocin mutations have been found in cases presenting at varying ages (32, 33). The reasons for this phenotypic variability are not yet completely understood, however, there is data supporting that the specific type or number of NPHS2 mutations may play a role (34, 35).

Perhaps the largest study to date on podocin mutations in sporadic cases best demonstrates the significant locus and allelic heterogeneity of NS. Using direct sequencing in 430 patients from 404 families from around the world, Hinkes, et al. was the first to document that patients with a specific type and number of mutations presented with symptoms at a significantly earlier age than patients without such mutations (33). Nevertheless, the authors did observe that identical mutations did result in different ages of onset within their study. Consequently, Hinkes, et al. concluded that there are likely to be additional modifying factors affecting the phenotype. It must also be noted that nearly 300 individuals in this study did not carry any mutation within the podocin gene, highlighting the likelihood of as yet undiscovered genes. This is the largest report to date, and the first to establish statistical significance of an important genotype-phenotype correlation. It serves as an example of the advantages of multi-national collaborations and demonstrates that further such studies are required in order to identify additional correlations with therapeutic and prognostic implications in the treatment of NS.

Currently, genetic screening is recommended for those children presenting with NS during their first year of life, as this could allow carriers of mutations in the NPHS1, NPHS2, LAMB2, and WT1 genes to be spared the potentially harmful treatments to which they have been shown not to respond (32, 36). Genetic testing may eventually become more widespread in children presenting with NS, regardless of age, as further studies establish links between currently known mutations and clinically-relevant phenotypic outcomes, as well as with the discovery of novel genes and variants. It should be noted that a different set of genetic variants may predispose to the steroid-sensitive form of NS (37), however, it has not been as intensively studied as SRNS due to its relatively benign clinical course. While there is currently much research focused on the genes and proteins of the slit diaphragm complex, further in-roads may be made in both SRNS and SSNS with the application of genome-wide association and expression studies to uncover novel susceptibility and modifying genetic variants. Such studies will require large-scale cohorts resulting from multi-institution and/or multi-national collaborations in order to further elucidate the pathogenetic mechanisms behind this rare and heterogenous syndrome.

Hemolytic Uremic Syndrome (HUS)

Hemolytic Uremic Syndrome (HUS) is similar to NS in that there are different manifestations of the disease and it appears to be genetically heterogenous. HUS occurs in its typical form following an infection associated with diarrhea, often from the E. Coli serotype 0157:H7. This form is relatively benign, and once resolved, complete renal function is generally restored. However, approximately 5–10% of HUS patients present with no previous infection. This atypical form, aHUS, can occur at any age, can be familiar or sporadic in nature and is associated with a poor clinical outcome, including a 25–30% mortality rate (38). While a number of environmental factors triggering aHUS have been reported (39–45), a genetic basis for this form has been suggested by familial cases (46–48). In 1998, the first aHUS-associated gene, complement factor H (CFH), was localized (49). Since then, aHUS has become largely recognized as a disease of complement alternative pathway dysregulation.

As with NS, some of the initial advances in understanding the etiology of aHUS were the result of genetic linkage and subsequent association studies. Using a candidate-gene linkage approach in three families, Warwicker et al. first demonstrated linkage of aHUS to a region on chr. 1 containing a number of genes involved in complement activation regulation (49). Subsequent mutational screening of the CFH gene identified two different mutations in one of the three families and in a sporadic aHUS patient. Since this first identification, numerous studies have reported various mutations within CFH associated with aHUS (49–54). Most mutations are missense mutations occurring in the C-terminal region of the protein; and functional analysis of CFH mutations show that they result in a reduced ability of CFH to protect cells from

complement lysis (55). Additional mutations within the complement regulatory genes of membrane co-factor protein (MCP) (56–58) and factor I (CFI) (59, 60), as well as within complement activating components factor B (CFB) (61) and C3 genes (62), have also been associated with aHUS. Together, these genes are estimated to account for approximately 50% of aHUS cases (39, 63–65). There is now an on-line database, www.fh-hus.org, which catalogues the various complement activation gene mutations associated with aHUS and provides a host of information regarding this disease and its genetic risk factors.

In order for complement gene mutation status to be translated into clinically useful information, a few reports have examined genotype-phenotype correlations in aHUS. Although each report is based on a small number of children, the results are consistent across studies. Carriers of CFH mutations have the most severe clinical course, demonstrating earlier age of onset, rapid progression to ESRD, and disease recurrence after kidney transplantation (63, 66). Those with MCP mutations show the most favorable prognosis, with no disease recurrence post-transplantation (39, 63, 66). CFI mutations carriers have an intermediate course, with 50% quickly progressing to ESRD, 50% recovering and a majority with disease recurrence post-transplantation (63, 66). These data demonstrate that aHUS presentation, response to treatment and long-term outcome are closely linked to genotype status. This should allow for more tailored management of children with aHUS and underscores the urgent need for new and improved therapies for those with CFH and IFH mutations.

An interesting feature of aHUS is its high rate of incomplete penetrance, approaching 50%, where disease-causing mutations are seen in those who do not present with the disease. It has been speculated that additional genetic factors may be required in addition to the known causative mutations in order for the disease to manifest itself. In addition, approximately half of aHUS patients do not carry a known causative mutation, suggesting as yet unidentified genetic variants. In this regard, a handful of polymorphisms, or SNPs, have also been associated with aHUS (50, 56, 65, 67,68). Caprioli et al. documented association of three SNPs in CFH with aHUS, concluding that they may predispose to the disease in those without CFH mutations, as well as contributing to the full manifestation of aHUS in CFH mutation carriers (50). Findings from Fremeaux-Bacchi, et al. support these data (56). A report by Esparza-Gordillo identified a SNP haplotype block spanning the complement activation gene cluster or chr.1q32 which was more frequent in aHUS patients, particularly those with CFH, MCP or F1 mutations, and which associated strongly with the severity of the disease (68). This same group later reported on a pedigree where three independent aHUS genetic risk factors were segregating, but only those members who inherited all three manifested aHUS (69). The authors hypothesize that multiple "hits" are required in complement activation genes in order to induce the aHUS phenotype. These data demonstrate the allelic heterogeneity and polygenic nature of aHUS.

Clearly, more work remains to be done, as only 50% of cases are explained by currently known mutations. It is likely that more genes and mutations await discovery. Moreover, the role of SNPs and their functional impact along side the currently known mutations needs to be better elucidated. As with NS, large-scale genomic association and expression studies may be helpful in this regard.

Chronic Kidney Diseases and End-Stage Renal Failure

As with the acute disorders, *Table 18-2* provides a summary of the genes, loci and genotype-phenotype correlations relevant to the below conditions.

Polycystic Kidney Disease (PKD) PKD represents a diverse collection of disorders of the tubules of the kidney, where healthy renal tissue is progressively replaced by fluid-filled cysts. Extra-renal manifestations are also seen in PKD, including hypertension, biliary ectasia and fibrosis leading to portal hypertension, hepatic cysts, abnormal heart valves, and intra-cranial aneurysms. Autosomal dominant PKD (ADPKD) is the most common form, with symptomatic onset usually during the third and fourth decades of life, although childhood onset has been documented (70, 71). Autosomal recessive PKD (ARPKD) is rarer, with symptoms often beginning *in utero* or during the neonatal period. Aside from palliative care, there are currently no known specific therapies for PKD. While the genetics of both ADPKD and ARPKD are well-established, additional advancements await through medical re-sequencing and the identification of genetic disease modifiers.

As with the aforementioned acute conditions, the genes for ADPKD, polycystin-1 (PKD1) and polycystin-2 (PKD2)/transient receptor potential channel (TRPP2), and the gene for ARPKD, fibrocystin (PKHD1), were identified through linkage analyses and subsequent positional cloning efforts (1–5). In ADPKD, the PKD1 gene accounts for approximately 85% of all families (72–74) and is associated with both a more severe clinical course and earlier onset (74–77). There is substantial allelic

heterogeneity of both PKD1 and PKD2, with most mutations unique to a family. The ADPKD database shows 314 and 91 likely pathogenic germ-line mutations within PKD1 and PKD2, respectively (http://pkdb.mayo.edu/; accessed 6/6/08).

As most mutations are private, genotype-phenotype studies must generally classify mutations in some way, usually based on type (missense v. truncating) and position within the protein. However, no strong correlations have yet been established between either type or position and phenotypic outcome for either gene. There is some evidence to suggest that patients with mutations in the 5' region of PKD1 progress to ESRD earlier and may be more susceptible to intra-cranial aneurysms (78, 79). While these two genes account for nearly all cases, the disease often presents with significant variability within families. While some of this variability is likely due to environmental factors, additional genetic factors are also suspected in modifying disease outcomes. Studies have estimated that 18–78% of the phenotypic variance in PKD1 and PKD2 populations may be attributable to genetic background (80–82). Many candidate gene studies have been performed in an attempt to identify some of these modifying factors; however, the results to date have been inconsistent (23, 83, 84). Because of the diversity of disease mutations and a lack of phenotypic correlations, the usefulness of genetic testing in ADPKD is limited, although it may be helpful in childhood cases with no previous family history. This may change as genotype-phenotype correlations and new therapies are eventually established.

In ARPKD, allelic effects play a greater role than in ADPKD. There are currently just over 300 mutations in the PKHD1 gene, according to the ARPKD database (http://www.humgen.rwth-aachen.de/; accessed 6/6/08). About 33% of mutations in this gene are unique to a single family (74). Although genotype-phenotype studies are limited by the substantial amount of allelic heterogeneity present within PKHD1, some conclusions can be drawn from these reports. In all studies to date, the presence of two truncating mutations has been shown to result in neonatal death, regardless of their position (85–87). All ARPKD patients which survive the neonatal period have been shown to carry at least one amino-acid substitution, as opposed to all chain-terminating mutations (85). While truncating mutations in general are associated with more severe disease, missense mutations have been shown to result in a milder phenotype (85). Larger cohorts of patients will be required to establish consistent and more detailed genotype-phenotype correlations that can be used to improve management of the disease, and in particular, to assist in the development of novel therapeutic approaches. As ARPKD is quite rare, and thus the numbers small, no candidate gene studies have been carried out to identify genetic modifiers, although some have been mapped in mouse models (74, 88).

In both ADPKD and ARPKD, the availability of fast, high-throughput medical re-sequencing, where patients are re-sequenced in-depth over long genomic regions in a matter of hours or days, offers a new opportunity for mutation detection, with a consequent increase in numbers of patients with specific mutational profiles. This should eventually aid in establishing clinically useful genotype-phenotype correlations. Moreover, the use of GWA studies in ADPKD, where the numbers are larger, may allow for the identification of novel genetic modifiers.

Primary IgA Nephropathy (IgAN) Primary IgAN is the most common form of glomerulonephritis in children worldwide, although its prevalence varies according to country (89–91). Some of this variance is attributable to geographic differences in the policies affecting renal biopsy in the young (92), while some may also be due to geographic differences in genetic and environmental factors (93, 94). Its incidence has been estimated as high as 25–30% in some Asian countries where children routinely undergo annual urinary screening, however, lower rates have been reported in Europe and the United States (95–98). IgAN is characterized by glomerular mesangial deposits of IgA and a widely-varying presentation (99). IgAN often presents with gross hematuria attendant to an upper respiratory tract infection (95, 98, 100, 101). However, it is estimated that in as many as half of all cases, there is only microscopic hematuria, which can continue for years before the development of proteinuria (95, 98, 101, 102). Because symptoms can be intermittent, and because in most countries there are no routine urinary screening programs, the diagnosis of IgAN can be missed.

IgAN was previously considered a benign disease, however, this outlook is changing based on the recognition of increased morbidity and mortality after long-term follow-up (95, 100, 103, 104), including the finding that approximately 10% and 20% of children progress to ESRD from ten and twenty years postdiagnosis, respectively (95, 100, 105–108). The disease progresses slowly over decades and with clinical symptoms often not presenting until years after onset, it is possible that many adult IgAN cases actually had a pediatric onset. Consequently, childhood IgAN may be considered an early stage of adult IgAN with life-long follow-up and evaluation required to detect the signs of disease progression (95). Enhanced detection of the disease in childhood may allow

for its earlier treatment and more aggressive management, offering a reduced risk of chronic kidney disease and ESRD at its later stages.

A complete understanding of the pathogenesis of IgAN is still lacking, although abnormally low glycosylation of IgAI molecules is a consistent finding in patients (109–111). Treatment of the disease is based upon symptoms, but ACE inhibitors are commonly prescribed due to their reno-protective effects. Some new drugs are currently under investigation, but more specific novel treatments are needed which can be used safely over the long-term in children. Obtaining a better understanding of this disease from a genetic standpoint should allow for insights into IgAN pathogenesis, as well as offering options for its improved diagnosis and treatment.

As with the diseases previously discussed, IgAN is genetically heterogenous, with familial aggregation identified in a subset of cases, while the bulk of cases present with seemingly sporadic onset. Currently, it is estimated that 15% of cases are "familial," however, it may actually be higher due to the difficulty in diagnosing the disease.

Within families, IgAN appears to follow an autosomal dominant inheritance pattern with incomplete penetrance. The most likely genetic model for IgAN resembles that of NS or aHUS, where there are a handful of primary susceptibility genes with additional modifying genetic and environmental factors also required. Yet the identification of genes for IgAN lags behind that of the acute kidney disorders, with no gene yet identified in familial cases. However, three genome-wide linkage studies have established a number of chromosomal regions linked to the disease. Gharavi, et al. identified a locus, termed *IGAN1*, on chromosome 6q22–23 as being linked to IgAN in 60% of the families from a Caucasian cohort (112). Using 22 informative Italian families from the IgAN biobank (113), Bisceglia, et al. reported linkage to *IGAN1* and also identified two suggestive loci on chromosomes 4q26–31 and 17q12–22(114). The most recent genome-wide linkage scan identified a locus on chromosome 2q36 in a Canadian family of Austrian-German descent with 14 affected subjects (115). These studies support the likely genetic heterogeneity of the disease. Review of the genes within these loci do not reveal likely candidates, however, this is not unexpected given the lack of understanding surrounding IgAN pathological mechanisms (116).

Not unlike the previous diseases, numerous smaller-scale case-control and family-based association studies have been undertaken for IgAN. Some of the genes studied to date, and for which significant associations have been reported, include the megsin gene, found in glomerular mesangium and upregulated in IgAN (117, 118); the uteroglobin gene, a multifunctional anti-inflammatory protein (119–121); and the C1GALT1 gene, an enzyme involved in the O-glycosylation process (122); and the E- and L- selectin genes, involved in endothelial and leukocyte cell-cell interactions, respectively (123). Associations have also been reported for a number of inflammatory candidates as well, including TNFα, TGFα, IL-4, IFγ, and HLA-DRA (124–127). In a partial genome-wide screen, a Japanese group has reported significant associations of the polymeric immunoglobulin receptor (PIGR) gene (128) and the immunoglobulin mu-binding protein 2 gene (129) using over 80,000 gene-based SNPs in a stepwise association design. Finally, while not associated with IgAN onset, a 32 bp deletion in the chemokine receptor 5 (CCR5) gene has been associated with increased renal survival in IgAN patients (130, 131). Results for many of the above genes require confirmation in larger, independent cohorts.

High-throughput genotyping of dense sets of SNPs within the currently known linkage peaks, now made possible through the HapMap Project and the commercial availability of mass-throughput SNP genotyping platforms, may assist in the identification of the genes responsible for the signals. GWA studies may also be useful and more economical in this regard. Once susceptibility genes are firmly established, the IgAN field can progress to identifying genotype-phenotype correlations to aid in diagnosis and treatment.

End-stage Renal Disease (ESRD) While many chronic kidney diseases can lead to ESRD, the causes of it differ between children and adults. Although hypertension and diabetes are the primary causes of ESRD in adult populations, FSGS and congenital structural kidney abnormalities are the primary causes in children. However, the well-documented worldwide increase in rates of childhood obesity, and its related complications of diabetes and hypertension, has the potential to significantly impact the rate of pediatric renal failure (132–137). The prevalence of ESRD in pediatric cohorts is generally less than that in adults, nevertheless, these children experience significant morbidity and mortality (138–140). In addition, ESRD disproportionately affects minorities, with African-American children having nearly double to triple the rates of Caucasian children within the same age categories (140).

Due to well-established statistics indicating a potentially catastrophic increasing public health burden of CKD and ESRD overall, the identification of genes related to these two outcomes represents a substantial opportunity for improvement in understanding the complex pathological mechanisms behind CKD progression.

A vast majority of the work in this area has been conducted in adult populations due to the higher prevalence of these outcomes, as well as to the availability of large cohorts of adults well phenotyped for the more common complex diseases which result in CKD and ESRD within this population. We will briefly review findings from the adult studies here, however, as the precipitating causes differ between adults and children, disease mechanisms leading to the end-point of ESRD may also differ. Therefore, genes identified in adults need to be tested for relevance in pediatric patients. Large, multi-institutional/multi-national cohorts of CKD and ESRD pediatric patients phenotyped in a standardized manner will be required to dissect the genetics of these complex and critical outcomes of various pediatric kidney diseases.

A number of groups have performed linkage analyses for ESRD in the context of one of its major precipitating causes in adults, diabetic nephropathy (DN) (141–153). Results from these linkage studies have identified numerous distinct loci, however, the best evidence supports DN susceptibility loci on chromosomes 10p and 18q (154). A susceptibility gene, carnosinase-1 (CNDP1), has been identified from the 18q locus. Two independent groups have demonstrated that diabetic patients harboring a particular leucine repeat polymorphism of CNDP1 are at a significantly reduced risk of developing nephropathy and ESRD (155, 156).

Although numerous association studies examining single genes have been published for ESRD, as in other cases, the small sample sizes and limited coverage of the candidate genes make results difficult to interpret (154). However, some larger-scale and more in-depth association scans, including a GWA study, have been published in this area and warrant discussion (15, 157, 158–160). In the first comprehensive, family-based screen of candidate genes for ESRD, Ewens identified twenty nominally significant genes, including twelve novel ones, in 72 type I diabetic trios (160). McKnight et al., used 6,000 microsatellites in 400 Irish patients with and without type I DN to identify two significant makers on chromosome 10, and four additional markers worthy of investigation on chromosomes 2, 4, 6, and 17 (159). A Japanese scan using over 80,000 SNPs in 87 type II diabetics with nephropathy and 92 diabetic controls without nephropathy identified the engulfment and cell motility 1 (ELMO1) gene as a DN susceptibility gene (158). In a recent GWA SNP association study, Hanson et al. found a significant association with the plasmacytoma variant translocation (PVT1) gene with ESRD in 207 type II diabetics with and without DN (157). Unfortunately, many of the above results still await verification (154).

It is noteworthy that the above studies all examined a qualitative outcome and were conducted in the setting of DN. However, there have been a number of both large-scale linkage and association studies published examining quantitative kidney function traits, such as glomerular filtration rate and urinary albumin excretion, in various populations (15, 141, 161, 162). Results from most of these studies require verification. A number of advantages have been suggested for the use of quantitative traits in the genetic dissection of complex diseases (163–165). The continued use of large and comprehensive linkage and association scans should assist in the further identification and replication of ESRD susceptibility loci. However, their relevance to children will have to be tested. The establishment of cohorts of children with ESRD is challenging given the small numbers, nevertheless, such cohorts can be collected through long-term collaborative efforts. Such cohorts are required to map genetic variation which may ultimately allow for more aggressive and tailored prevention and treatment efforts in this life-threatening disorder.

The Past, Present and Future Application of Genomic Methods in Pediatric Nephrology

With the application of genome-wide linkage analyses as one of the first genomic technologies, pediatric nephrology witnessed some of the first fundamental advances in the understanding of many chronic and acute kidney diseases. With the completion of the HGP and the availability of genome-wide SNP markers that were easily and inexpensively typed, the field witnessed the candidate-gene era, where one or a few genes were genotyped for a handful of SNPs in usually small to moderate size samples with inadequate statistical power for detecting true disease associations. The popularity of this method was due in large part to its ease of application; however, the current and widespread use of candidate gene studies has limited ability to result in the novel breakthroughs required to enable paradigm changes in the prevention, diagnosis and treatment of these often life-threatening conditions. In order for the field to take the next step in the understanding of such diseases, the application of the next generation of genomic technologies to pediatric renal diseases in required, including GWA studies with hundreds of thousands to millions of SNPs. In order for these studies to become a reality within the field, large, well-phenotyped cohorts of children are necessary. The sample sizes needed for GWA studies to be successful, those samples approaching

1,000 or more, cannot be collected at a single center due to the often modest number of cases of any particular kidney disorder. Consequently, it becomes apparent that investigators need to form inter-institutional and even international collaborations with standardized methods of phenotyping. This is being pursued to some extent with adult renal patients and it is just beginning in the pediatric case. More collaborations can be expected as the importance of genomic technologies in pediatric nephrology is increasingly recognized, as such technologies become more readily available to investigators and as the infrastructure required for such studies becomes more firmly established. GWA studies offer a hypothesis-free design to gene discovery and are therefore ideal for many complex kidney diseases where there is limited knowledge of disease mechanisms. Their analysis, in conjunction with the other genome-wide technologies of transcriptomics and proteomics, offers the best hope for future fundamental advances in the field of pediatric nephrology.

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