The expanding phenotypic spectra of kidney diseases: insights from genetic studies

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Abstract | Next-generation sequencing (NGS) has led to the identification of previously unrecognized phenotypes associated with classic kidney disease genes. In addition to improving diagnostics for genetically heterogeneous diseases and enabling a faster rate of gene discovery, NGS has enabled an expansion and redefinition of nephrogenetic disease categories. Findings from these studies raise the question of whether disease diagnoses should be made on clinical grounds, on genetic evidence or a combination thereof. Here, we discuss the major kidney disease-associated genes and gene categories for which NGS has expanded the phenotypic spectrum. For example, *COL4A3–5* genes, which are classically associated with Alport syndrome, are now understood to also be involved in the aetiology of focal segmental glomerulosclerosis. *DGKE*, which is associated with nephrotic syndrome, is also mutated in patients with atypical haemolytic uraemic syndrome. We examine how a shared genetic background between diverse clinical phenotypes can provide insight into the function of genes and novel links with essential pathophysiological mechanisms. In addition, we consider genetic and epigenetic factors that contribute to the observed phenotypic heterogeneity of kidney diseases and discuss the challenges in the interpretation of genetic data. Finally, we discuss the implications of the expanding phenotypic spectra associated with kidney disease genes for clinical practice, genetic counselling and personalized care, and present our recommendations for the use of NGS-based tests in routine nephrology practice.

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The introduction of next-generation sequencing (NGS) has revolutionized the field of genetics over the past ten years. Rapidly decreasing costs and the increasing speed and availability of NGS technologies have paved the way for routine genetic testing in nephrogenetic diseases such as polycystic kidney disease[1,2] and familial haematuric nephropathies[3,4]. NGS has increased the applicability of genetic testing in genetically heterogeneous kidney diseases such as nephrotic syndrome[5] (OMIM 256300) and has provided a time and cost-efficient strategy to aid the diagnosis of diseases that are technically challenging to diagnose from a genetics standpoint, such as autosomal dominant polycystic kidney disease (ADPKD; OMIM 173900), for which multiple pseudogenes exist[1]. NGS has also boosted gene discovery in numerous kidney disease categories including renal ciliopathies[6], nephrotic syndrome[7,8], focal segmental glomerulosclerosis[9] (FSGS; OMIM 603278) and congenital anomalies of the kidney and urinary tract (CAKUT; OMIM 610805)[10,11]. A monogenic cause can now be identified in approximately 20% of patients with early-onset chronic kidney disease (CKD), depending on the population structure[12]. In addition to enabling faster and more comprehensive molecular testing for genetic kidney diseases, NGS also has potential for clinical application through urinary biomarker-based diagnosis of renal tubular injury[13], for tumour classification and pharmacogenomics in onconephrology[14,15] and for histocompatibility assays in renal transplantation[16].

In both research and diagnostic settings, NGS is evolving from an approach used to sequence panels of known and candidate kidney-disease associated genes (gene panel sequencing), to whole-exome sequencing and whole-genome sequencing to identify causal disease-associated genes[17]. The progression of NGS towards increasingly untargeted approaches has resulted in the emergence of surprising findings, leading to reclassification of clinical diagnoses and broadening our

Key points

- Findings from next-generation sequencing (NGS) have led to a shift in phenotypic boundaries and reclassifications of some kidney diseases
- NGS techniques are a valuable addition to the diagnostic toolbox in nephrology and findings from NGS can have important implications for therapeutic strategies and clinical outcomes
- Interpretation of genetic variants and accurate prediction of the associated kidney phenotype can be challenging despite the increasing availability of bioinformatics tools and functional tests
- Data sharing initiatives are imperative to establish clinically useful genotype–phenotype correlations and to maximize the benefit of genetic testing in routine nephrology practice

understanding of the phenotypic spectrum of classic kidney disease-associated genes. Reclassification of clinical diagnoses on the basis of molecular findings from NGS is particularly relevant for renal diseases such as ADPKD[18] and nephronophthisis[19] (OMIM 256100), for which phenotypically similar conditions exist. For diseases such as these, the identification of disease-causative genes has the potential to result in an unequivocal diagnosis.

In cases where a clinical diagnosis is unequivocal, findings from NGS have led to improved insights into phenotypic variability related to mutations in known kidney disease-associated genes. Clinical phenotypes with genetic overlap can exist within the same disease category, for example renal ciliopathies. However, phenotypes can also be clinically unrelated; for example mutations in *DGKE* can cause both nephrotic syndrome type 7 with membranoproliferative glomerulonephritis (MPGN; OMIM 615008) and atypical haemolytic-uraemic syndrome (aHUS; OMIM 235400), diseases that are considered clinically unrelated[20–22]. In these and other apparently distinct phenotypes, shared genetic aetiology can provide insight into common underlying pathophysiological processes.

In this Review, we discuss examples of genes and gene categories in which NGS has led to an expansion in our understanding of the phenotypic spectrum, both across and within current kidney disease categories (TABLE 1). We examine how these findings can provide insight into gene function and underlying pathophysiological mechanisms, and consider the genetic and epigenetic factors that contribute to kidney disease heterogeneity. Finally, we highlight the implications of broader phenotypic spectra of classic kidney disease-associated genes for clinical practice, genetic counselling and personalized medicine, and present our current approach for the use of NGS-based tests in routine nephrology practice.

Expanding renal disease phenotypes

Expansions across disease categories

COL4A3–5 mutations in Alport syndrome and FSGS. NGS has indisputably broadened the phenotypic spectrum of diseases associated with mutations in *COL4A3–5*, which encode the α-chains of glomerular basement membrane collagen type IV. Mutations in these genes are classically associated with Alport syndrome (OMIM 301050), a glomerulonephropathy associated with variable sensorineural hearing loss and ocular anomalies. Clinically, Alport syndrome is characterized by microscopic haematuria with proteinuria and, eventually, renal failure[23]. Inheritance of Alport syndrome is X-linked in 65% of cases (only in patients with mutations in *COL4A5*) and dominant (20%) or recessive (15%) in other cases (that is, in patients with mutations in *COL4A3* and *COL4A4*)[23]. Several studies have shown the practical benefits of NGS in the diagnostic workup of patients with Alport syndrome[24,25].

The allelic disease thin basement membrane nephropathy (TBMN; OMIM 141200) is also characterized by persistent microscopic haematuria. Contrary to Alport syndrome, however, TBMN is rarely reported in combination with progressive proteinuria and end-stage renal disease (ESRD), although this milder phenotype might reflect a lack of longitudinal studies of patients with TBMN[23,26–28]. Studies from 2007 and 2009 described families with TBMN caused by heterozygous mutations in *COL4A3* and *COL4A4* (REFS 27,28). In these studies, nearly all patients with microhaematuria, proteinuria and CKD showed FSGS on examination of renal biopsy samples, suggesting that specific heterozygous mutations in *COL4A3/4* or unknown genetic modifiers might cause concurrent FSGS lesions in addition to TBMN[27,28].

Later studies using NGS identified mutations in *COL4A3–5* genes in patients who had a primary diagnosis of FSGS but, on closer clinical evaluation, exhibited features of Alport syndrome, such as characteristic findings on electron microscopy and hearing loss[29–31]. In addition, targeted sequencing of 26 glomerular genes in 50 patients with steroid-resistant nephrotic syndrome (SRNS) and/or FSGS identified three patients with mutations in genes known to cause SRNS and/or FSGS in combination with a heterozygous mutation in *COL4A3*, suggesting a modifier effect of *COL4A3* that might aggravate the phenotype of SRNS and/or FSGS[32].

NGS has also led to the detection of *COL4A* variants in patients with isolated FSGS. For example, heterozygous missense *COL4A3* variants were identified in five of 40 Chinese families with hereditary FSGS and one of 50 Chinese patients with sporadic FSGS[33]. *COL4A3* and *COL4A4* missense and truncating mutations were also identified in seven of 70 families with a primary diagnosis of FSGS accompanied by proteinuria and haematuria. Clinical and pathological findings were inconsistent with Alport syndrome in four of these families[34]. Finally, *COL4A3–5* mutations were detected in 38% of families with FSGS ($n = 8$) and in 3% of patients with sporadic FSGS ($n = 67$)[35]. Although one patient with familial FSGS developed hearing loss consistent with Alport syndrome, the researchers claimed that their findings show that *COL4A3–5* mutations are the most frequent cause of FSGS in adults[35].

Whether the above-described cases of FSGS should be considered to be familial FSGS or rather a secondary effect of undiagnosed TBMN is a matter of debate[36]. FSGS occurs in at least 5% of patients

Next-generation sequencing
A technique that enables the simultaneous investigation of mulitple genes and pathways in parallel. The term includes all forms of modern, high-throughput sequencing techniques, including gene panel sequencing, whole-exome sequencing and whole-genome sequencing.

Nephrogenetic diseases
Kidney diseases with a genetic aetiology, including hereditary kidney disorders for which the responsible genes have not yet been identified.

Pseudogenes
DNA sequences that are similar to genes but do not encode functional proteins.

Gene panel sequencing
Targeted sequencing of a set of genes that are associated with a specific phenotype.

Whole-exome sequencing
Targeted sequencing of all the protein-coding regions (1–2%) of the genome.

Whole-genome sequencing
Untargeted sequencing of the complete genome.

Causal
Variant(s) that are the cause of a specific phenotype.

Missense
A variant that results in a single amino-acid substitution.

Truncating
A variant that introduces a premature stop codon and results in a shortened protein.

Table 1 | **Summary of genes discussed in this Review**

Gene	Associated diseases (mode of inheritance*)	Potential mechanisms
Across disease categories		
COL4A3–5	FSGS (AD)[33–35] TBMN (AD)[122,123] Alport syndrome (AD, AR, XL)[124,125]	FSGS could arise secondary to unrecognized TBMN through complex mechanisms involving cross-talk between components of the glomerular filtration barrier, through defects in the GBM that result in podocyte foot-process effacement and GBM scarring, and through the actions of modifier genes[36,38]
DGKE	MPGN (AR)[39] aHUS (AR)[20–22,40]	• aHUS could arise secondary to MPGN and *vice versa* because loss of *DGKE* function and complement activation both cause sustained diacylglycerol signalling[39,44], which induces glomerular epithelial cell injury, proteinuria, upregulation of prothrombotic factors and platelet activation[20,44,46,47], and increases TRPC6 channel activity, which is associated with podocyte foot-process effacement and nephrotic syndrome[44,48] • In *DGKE*-mediated aHUS, the main prothrombotic effect is mediated through upregulation of the pro-inflammatory p38–MAPK pathway that causes impaired endothelial cell proliferation and angiogenesis[45]
TTC21B	Ciliopathies (AR)[51] Glomerulopathies (AR)[52,53]	• Most patients with *TTC21B*-related glomerulopathy reported to date carry biallelic p.Pro209Leu mutations. This mutation has also been identified in patients with nephronophthisis[51–53] • Glomerulopathies could result from *TTC21B*-mediated alterations in cytoskeletal architecture in mature podocytes, which affects cell size and migration, actin and microtubule networks and cell nucleation[52] • Tubular lesions characteristic of nephronophthisis suggest a concomitant ciliary defect in glomerulopathy patients[52,53]
PAX2	RCS (AD)[126] CAKUT (AD)[127] FSGS (AD)[128]	• Truncating *PAX2* mutations are more frequently associated with RCS, while missense mutations are more frequently associated with isolated CAKUT or FSGS[128] • Location of the mutation within the gene is associated with the phenotype[128] • FSGS could develop secondary to subtle renal developmental anomalies or through dysregulation of PAX2 targets (for example WT1) involved in podocyte development and/or function[128]
HNF1B	RCAD (AD)[129] CAKUT (AD)[130,131]	• *HNF1B* is involved in regulation of renal tubular epithelial cell proliferation and differentiation, and expression of cystic kidney disease-associated genes[132–134] • There are no clear genotype–phenotype correlations in *HNF1B*-related renal disease[135]
Within disease categories		
MKS1, *B9D1*, *CEP290*	MKS and JBTS (AR)[54,136–139]	• Both *MKS1*-related INPP5E mislocalization and mutations in *INPP5E* can cause Joubert syndrome, suggesting that INPP5E dysfunction is central to the pathogenesis of Joubert syndrome[55–57] • In MKS, a complete loss of MKS1 leads to a marked defect in cilia formation and could result in more severe mislocalization of ciliary proteins[55]
WT1	Wilms tumour, DDS and SRNS (AD, SM)[140–142]	Mutation type and location within the gene affect the risks of Wilms tumour and SRNS[63,65]. The exact mechanisms are unclear
Within phenotypes		
HNF4A	MODY1 and Fanconi renal tubular transport disorder (AD)[67,68,143]	HNF4A is a known regulator of *SLC2A2*, which encodes the glucose transporter GLUT2, and mutations in *SLC2A2* cause Fanconi–Bickel syndrome, suggesting that defective HNF4A could lead to impaired renal tubular transport[67,68]
LMX1B	NPS and glomerulopathy (AD)[69–71,144]	Mutations associated with an isolated glomerulopathy are located in the homeodomain of *LMX1B*, possibly disrupting the interaction between LMX1B and (podocyte specific) DNA targets[69–71]. However, mutations in this domain have been reported in NPS patients as well[145]
OCRL1	Lowe syndrome and Dent disease (XL)[72,146]	• Phenotypic variability could arise from different locations of mutations within *OCRL1*, differential splicing of *OCRL1* resulting in different isoforms and the presence of genetic modifiers[147,148] • Pathophysiological mechanisms hypothesized to underlie Lowe syndrome and Dent-2 disease include cilia and cell polarity defects, and aberrant endocytic trafficking[149]
FRAS1, *FREM2* *GRIP1*	Fraser syndrome and CAKUT (AR)[74,150–152]	• Biallelic truncating mutations cause Fraser syndrome, while biallelic missense mutations are associated with isolated CAKUT[74] • Biallelic missense mutations could result in a partial loss-of-function, which might be compensated for in the development of extrarenal tissues but not in development of the kidneys[74]

AD, autosomal dominant; aHUS, atypical haemolytic uraemic syndrome; AR, autosomal recessive; CAKUT, congenital anomalies of the kidney and urinary tract; DDS, Denys–Drash syndrome; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; JBTS, Joubert syndrome; MKS, Meckel–Gruber syndrome; MODY1, maturity-onset diabetes of the young type 1; MPGN, membranoproliferative glomerulosclerosis; NPS, nail–patella syndrome; RCAD, renal cysts and diabetes syndrome; RCS, renal-coloboma syndrome; SM, somatic mutation; SRNS, steroid-resistant nephrotic syndrome; TBMN, thin basement membrane nephropathy; XL, X-linked. *Inheritance pattern is provided in the context of the affected gene. Sequencing techniques such as whole-exome sequencing have expanded the phenotypic spectrum associated with kidney disease genes. The subgroups contain selected examples from this Review.

with TBMN[37]. TBMN could potentially lead to secondary FSGS through complex mechanisms involving crosstalk between components of the glomerular filtration barrier, through defects in the glomerular basement membrane (GBM) that result in podocyte foot-process effacement and GBM scarring, or through the actions of modifier genes, such as *NPHS2* (REFS 36,38). In the studies described above[33–35], most patients were diagnosed with FSGS well into adulthood; the possibility of these patients having secondary FSGS cannot, therefore, be excluded[38]. As an erroneous diagnosis of primary FSGS can lead to inaccurate counselling and unwarranted corticosteroid treatment[36], the value of redefining the *COL4A* phenotypic spectrum to include both Alport syndrome and FSGS warrants serious discussion[34]. An alternative proposal is to redefine the Alport spectrum by including benign familial haematuria and TBMN (caused by heterozygous *COL4A3/4* mutations) at the mild end, secondary FSGS and late ESRD in the middle and early ESRD with extrarenal features (caused by biallelic *COL4A3/4* mutations or hemizygous *COL4A5* mutations) at the severe end of the spectrum[38] (FIG. 1). Large, genetic studies with longitudinal follow-up are required to confirm genotype–phenotype correlations associated with *COL4A* mutations, which will only realistically be achieved through collaborative multicentre networks.

***DGKE* mutations in MPGN and aHUS.** Homozygous truncating mutations in *DGKE*, which encodes diacylglycerol kinase ε (DGKε), were first identified by whole-exome sequencing in a Turkish family with nephrotic syndrome type 7 with MPGN-like glomerular microangiopathy, and, subsequently, in two other families with MPGN[39]. Histologically, biopsy samples from these patients exhibited hypertrophic, hyperlobulated and hypercellular glomeruli, thickening and splitting of the basement membrane, podocyte foot-process effacement and swelling of endothelial cells with obstruction of the capillary lumen[39].

Whole-exome sequencing also revealed biallelic missense and truncating mutations in *DGKE* in two families with childhood-onset aHUS. *DGKE* mutations were subsequently identified in six of 47 additional unrelated probands who also had childhood-onset aHUS[20]. In addition, a study using whole-genome sequencing identified a biallelic intronic *DGKE* mutation that segregated with paediatric-onset aHUS in two unrelated families. Subsequent cDNA sequencing showed that the intronic mutation causes aberrant splicing of the *DGKE* transcript, which is predicted to alter the protein catalytic domain that phosphorylates diacylglycerol[21]. In a study of 83 patients with early-onset aHUS, four patients had biallelic *DGKE* mutations, of whom three showed additional heterozygous mutations in the genes encoding thrombomodulin (*THBD*) and complement component C3 (REF. 40). Finally, homozygous truncating mutations in *DGKE* were also reported in two of 1,783 families with SRNS[41].

aHUS can develop secondary to MPGN and *vice versa*. For example, patients with a primary diagnosis of MPGN caused by mutations in genes that regulate the alternative complement pathway (for example *CFH* and *C3*), can develop secondary aHUS[42,43]. Multiple mechanisms link *DGKE* mutations to podocyte dysfunction, complement activation and thrombotic microangiopathy[44,45]. DGKε phosphorylates diacylglycerol and is expressed in the endothelium of glomerular capillaries and podocytes[20,39]. Both loss of DGKε function and complement activation cause sustained diacylglycerol signalling[39,44], which induces glomerular epithelial cell injury, proteinuria, upregulation of prothrombotic factors and platelet activation[20,44,46,47]. Furthermore, sustained diacylglycerol signalling increases TRPC6 channel activity, which is associated with podocyte foot-process effacement and nephrotic syndrome[44,48]. Based on the above-described mechanisms, one could expect that aHUS could develop during the course of *DGKE*-associated MPGN or could even precede the manifestations of MPGN. However, in the above-described families with MPGN caused by *DGKE* mutations, no signs of aHUS preceded the onset of MPGN, rendering it unlikely that MPGN occurred secondary to aHUS[39].

Strikingly, most of the families with aHUS caused by *DGKE* mutations without additional mutations in *THBD* or *C3* did not show abnormal complement activation[20,21,40], and patients who received anticomplement therapy (eculizumab or plasma infusion) experienced an acute episode of aHUS while on treatment, suggesting the presence of a complement-independent mechanism with major implications for treatment[20]. Studies into the mechanism of *DGKE*-associated aHUS showed that, although *DGKE* knockdown moderately downregulates expression of the complement inhibitory protein membrane cofactor protein (MCP), no increase in endothelial C3 deposition occurs[45]. Rather, the main prothrombotic effect is mediated through DGKε-mediated upregulation of the proinflammatory p38–MAPK pathway with impaired endothelial cell proliferation and angiogenesis that occurs in a complement-independent manner[45]. However, exceptions to this mechanism of action exist; a report of a family with *DGKE*-related aHUS with substantial serum complement activation and apparent responsiveness to plasma infusion therapy suggests that some patients with *DGKE*-associated aHUS can benefit from complement-blocking agents[22]. Alternatively, the activation of serum complement in these patients could be the result of a transient effect of microparticles that are released from apoptotic endothelial cells and that augment DGKε-mediated damage[45,49]. Although this last possibility suggests that *DGKE* mutations might not preclude efficacy of complement blocking therapy, it does illustrate the value of a pharmacogenomic perspective in the management of patients with hereditary kidney disease.

***TTC21B* mutations in ciliopathies and glomerulopathies.** *TTC21B* encodes the ciliary protein tetratricopeptide repeat domain-containing protein 21B (also known as IFT139), which is required for retrograde intraflagellar transport[50]. Using targeted sequencing of *TTC21B* in a cohort of 753 patients with

Biallelic
Variant present on both alleles of a specific gene. Biallelic variants can be homozygous or compound heterozygous.

Probands
Patients who are the starting points of genetic studies in families.

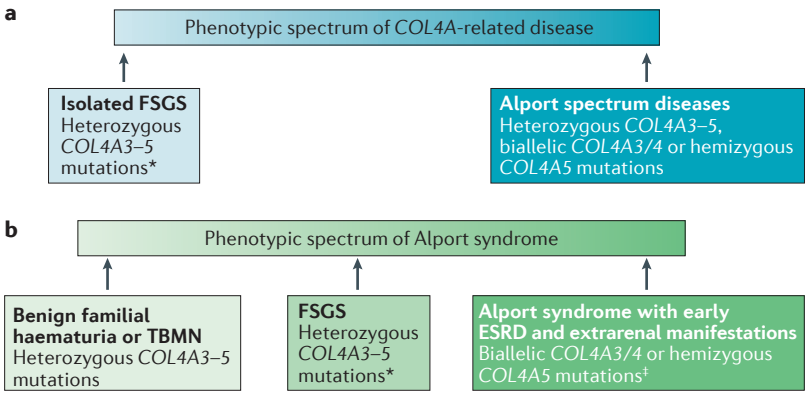

Figure 1 | **The phenotypic spectrum of *COL4A3–5* mutations.** Two proposals exist as to how *COL4A* mutations that cause focal segmental glomerulosclerosis (FSGS) might be considered in the classical Alport paradigm. The first proposal (a) is to revise the phenotypic spectrum of *COL4A*-related disease to include both isolated FSGS and Alport syndrome-related phenotypes (including benign familial haematuria, thin basement membrane nephropathy (TBMN) and Alport syndrome). The second (b) proposes that FSGS exists as part of the phenotypic spectrum of Alport syndrome because it cannot be excluded that FSGS occurs secondarily to (as yet) undiagnosed TBMN or Alport syndrome[38]. *Hemizygous *COL4A5* missense mutations and biallelic *COL4A3* mutations have also been described in patients with FSGS[34,35]. The degree of pathogenicity of hemizygous *COL4A5* missense mutations was not functionally assessed and mutations could therefore be hypomorphic. ‡Women with heterozygous mutations in *COL4A5* can develop end-stage renal disease and/or hearing loss depending on (tissue specific) X-chromosome inactivation[153].

diverse ciliopathies, biallelic mutations in *TTC21B* were identified in five families with nephronophthisis (OMIM 613820) and in one family with another ciliopathy, Jeune asphyxiating thoracic dystrophy (OMIM 613819)[51]. In addition, heterozygous pathogenic missense and truncating variants in *TTC21B* were found in 5% ($n = 38$) of the cohort. One-third of these mutations were found in combination with biallelic mutations in other known ciliopathy-associated genes. Although rare variants of *TTC21B* were not enriched in patients with ciliopathies compared to controls, pathogenic variants were, however, significantly enriched in such patients, suggesting a modifier role of *TTC21B* in ciliopathies[51].

The association between pathogenic *TTC21B* variants and ciliopathies is not surprising given the known role of *TTC21B* in regulating ciliary function; however, whole-exome sequencing also identified homozygous missense mutations in *TTC21B* in seven of 46 families with a primary diagnosis of late-onset FSGS[52]. The p.Pro209Leu mutation identified in these families was previously reported in patients with nephronophthisis[51]. In addition, biallelic mutations in *TTC21B* (p.Pro209Leu, p.His426Asp) were identified in three of 15 families with childhood-onset nephrotic proteinuria, renal biopsy findings of FSGS and tubulointerstitial lesions[53]. Although secondary glomerulosclerosis has been observed in patients with advanced nephronophthisis, these reports constitute the first evidence of a ciliary gene being involved in a hereditary glomerulopathy.

The p.Pro209Leu mutation has a hypomorphic effect on cilia formation in undifferentiated podocytes; however, the glomerular defects seem to be mediated by a non-ciliary effect of the mutation on cytoskeletal architecture in mature podocytes, which affects cell size and migration, as well as actin and microtubule networks and cell nucleation[52]. Histological examination of biopsy samples from patients with *TTC21B*-associated FSGS showed tubular lesions characteristic of nephronophthisis in addition to classic FSGS lesions, suggesting a concomitant ciliary defect in renal tubular epithelial cells[52,53]. Other ciliary genes that function in the podocyte cytoskeleton, for example the glycogen synthase kinase 3β, might give rise to primary FSGS as well, providing a molecular link between tubulointerstitial and glomerular phenotypes.

Expansions within disease categories

Phenotypic heterogeneity in ciliopathies. The most striking example of a situation in which NGS findings have challenged the categorization of renal diseases is within ciliopathies, in which mutations in *MKS1* and *B9D1*, previously associated with the embryonic lethal Meckel–Gruber syndrome (OMIM 249000), were identified in patients with the viable and less severe Joubert syndrome (OMIM 213300), indicating that these syndromes represent two ends of a phenotypic spectrum[54]. Biallelic truncating mutations in *MKS1* cause Meckel–Gruber syndrome; the presence of at least one hypomorphic missense mutation leads to Joubert syndrome due to malfunction of MKS1 at the ciliary transition zone at the base of cilia and a subsequent reduction in ARL13B and INPP5E inside the cilium[55]. Both *MKS1*-related INPP5E mislocalization and mutations in *INPP5E* can cause Joubert syndrome, suggesting that INPP5E dysfunction is central to the pathogenesis of this disease[55–57]. In Meckel–Gruber syndrome, the complete loss of MKS1 leads to a marked defect in cilia formation and could result in severe mislocalization of ciliary proteins[55]. This example is characteristic of the phenotypic variability seen with mutations in ciliopathy-associated genes[58,59]. Nonsense-associated alternative splicing, a mechanism whereby detrimentally mutated exons are spliced out of the final gene transcript, partially explains phenotypic variability in patients with *CEP290*-related ciliopathies[60,61] (FIG. 2). This mechanism of basal exon skipping is expected to extend beyond ciliopathies and apply to other diseases with considerable phenotypic heterogeneity as well[60]. In addition to variations in the degree of protein impairment, genetic modifiers, such as heterozygous mutations in *TTC21B*[51], also contribute to phenotypic heterogeneity in ciliopathies[62].

Phenotypic variability in WT1-related syndromes. Similar to ciliopathies, mutation type determines phenotypic outcome in *WT1*-related syndromes. In a cohort of 117 patients with Wilms tumour (OMIM 194070) associated with *WT1* mutations, truncating mutations were associated with the highest risk of early-onset bilateral Wilms tumour, followed by missense mutations and then deletions, which were most frequently associated with a syndromic phenotype[63]. Missense mutations in exon 8 and exon 9 were associated with Denys–Drash syndrome (OMIM 194080), which is

Pathogenic
Variant that has an effect on protein function that is associated with a specific disease phenotype.

Hypomorphic
A mutation that results in reduced expression or reduced activity of a protein. The resulting disease phenotype is potentially milder than when mutations cause a complete loss of functional protein.

Phenotypic heterogeneity
When mutations in the same gene can give rise to two or more distinct clinical phenotypes.

typically associated with early-onset, unilateral Wilms tumour[63,64]. In a cohort of 61 patients with *WT1*-related SRNS, truncating mutations were associated with a high risk of developing Wilms tumour (78%) and late-onset SRNS. Conversely, missense mutations were associated with diffuse mesangial sclerosis and early-onset SRNS and ESRD, and intronic (exon 9 splice site) mutations were associated with early-onset SRNS, FSGS on renal biopsy and a very low tumour risk[65]. This example also illustrates that isolated presentations, such as early-onset SRNS or genitourinary anomalies, can be indicative of a tumour predisposition syndrome, which is relevant for genetic counselling[66].

Different presentations of the same phenotype

Some disease phenotypes differ only by the presence of renal or extrarenal features. This phenotypic heterogeneity can be caused by the inheritance pattern of the causative gene, the mutation type, the presence of genetic modifiers or other, often unknown, factors. Contrary to expanding phenotypes across and within disease categories, patients with different presentations of the same phenotype share core (for example, extrarenal) features. For instance, a heterozygous missense mutation in *HNF4A*, associated with maturity-onset diabetes of the young (MODY) type 1 (OMIM 125850) without renal involvement, has also been identified in patients with primary Fanconi renal tubular transport disorder (OMIM 616026) co-presenting with MODY[67,68]. HNF4A is a known regulator of *SLC2A2*, which encodes the glucose transporter GLUT2, and mutations in *SLC2A2* cause Fanconi–Bickel syndrome (OMIM 227810), suggesting that defective HNF4A could lead to impaired renal tubular transport[67]. We expect that *HNF4A* mutations in patients with primary Fanconi renal tubular transport disorder and MODY will be identified more frequently with increasing use of NGS, which will confirm the role of *HNF4A* in this phenotype.

Finally, different inheritance patterns or mutation types in genes involved in syndromic kidney disease can cause an isolated kidney phenotype at the mild end of the phenotypic spectrum. Examples are mutations in *LMX1B*, which are associated with nail–patella syndrome (OMIM 161200) but can also cause an isolated glomerulopathy[69–71], and mutations in *OCRL1*, which cause Lowe oculocerebrorenal syndrome (OMIM 309000), but also cause Dent disease 2 (OMIM 300555)[72]. Another example is provided by *FRAS1* and *FREM2*, in which biallelic truncating mutations cause Fraser syndrome (OMIM 219000). A 2012 study identified heterozygous missense mutations in *FRAS1* and *FREM2* in patients with isolated unilateral renal agenesis[73]; however, a more extensive study by the same group in 2014 showed that biallelic missense mutations, rather than heterozygous missense mutations, in six genes associated with the Fraser–MOTA (manitoba-oculo-tricho-anal)–BNAR (bifid nose with or without anorectal and renal anomalies) spectrum of diseases, including *FRAS1* and *FREM2*, were responsible for 2.2% cases of CAKUT in 590 affected families[74]. Understanding exactly how these disease phenotypes correlate with inheritance and mutation type can have important consequences for diagnosis, clinical management and genetic counselling.

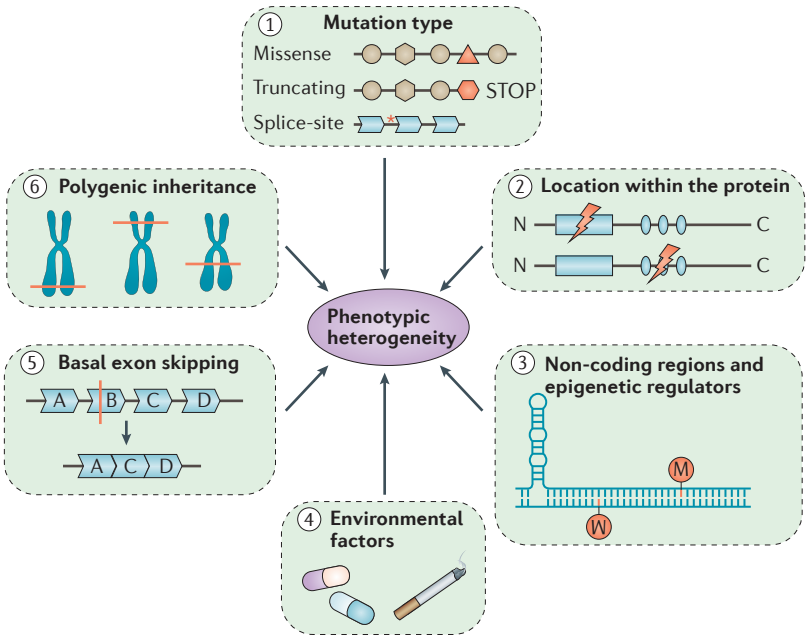

Figure 2 | **Sources of phenotypic heterogeneity in nephrogenetic disease.** In general, truncating and splice-site mutations are expected to be more detrimental to protein function than are missense mutations (1). The phenotype can vary according to the location of the mutation within the protein. For example, mutations in important functional protein domains are more likely to affect protein function than are mutations outside functional domains (2). The expression of genes is regulated by noncoding factors such as microRNAs and by epigenetic modifications such as methylation (3). Environmental factors such as nutrition and medication have a role in the expression of multifactorial phenotypes (4). Basal exon skipping is a mechanism by which severely mutated exons can be selectively spliced out of the gene transcript. The resulting shortened transcript escapes nonsense-mediated decay and can retain partial function if the skipped exon was outside an important functional domain (5). Variants in multiple genes can influence the type and severity of phenotypic features. Modifier variants can have an additive pathogenic or a protective effect (6).

Reclassifying kidney disease

Genetic findings from NGS can also lead to reclassification of kidney disease in individual patients. For example, patients with Alport syndrome have been misdiagnosed as having MPGN and patients with congenital chloride diarrhoea caused by *SLC26A3* mutations have been misdiagnosed as having Bartter syndrome[75,76]. Clinical phenotyping can confirm a molecular diagnosis by revealing additional symptoms such as hearing loss and retinal anomalies in a patient with Alport syndrome, illustrating the importance of 'reverse phenotyping' (REF. 75).

Genetic diagnostics can aid clinical phenotyping especially for diseases with variable or incomplete phenotypic expression. For example, missense and truncating mutations in *CLCN5* were detected in patients presenting with a Bartter-like phenotype (OMIM 601678) in whom mutations in known disease-associated genes were excluded[77–79] and in patients with focal segmental and/or global glomerulosclerosis[80–84].

CLCN5 encodes the voltage-gated chloride channel ClC-5, which is classically associated with Dent disease (OMIM 300009). Dysfunctional *CLCN5* is hypothesized to disrupt the endocytosis pathway in the proximal tubule and, consequently, increased sodium delivery to the distal tubule might induce hypovolemia, thereby activating the renin–angiotensin–aldosterone axis to cause Bartter-like features of hypokalaemic metabolic alkalosis, hypercalciuria and hyper-reninaemic hyperaldosteronism[77,78], even though the clinical presence of low molecular weight proteinuria excluded a diagnosis of Bartter syndrome. Similarly, patients who presented with nephrotic range proteinuria and focal segmental and/or global glomerulosclerosis had selective albuminuria with normal serum albumin level, an absence of oedema, and minimal podocyte foot-process effacement on renal biopsy, suggesting a tubular rather than a glomerular aetiology and a missed diagnosis of Dent disease, which was confirmed by genetic findings[80–84]. A diagnosis of Dent disease in patients with proteinuria has major consequences for treatment because immunosuppressive therapy, which can have considerable adverse effects, can be discontinued. In a patient with SRNS and a hemizygous truncating *CLCN5* mutation, treatment was changed from immunosuppressive therapy to an angiotensin-converting-enzyme (ACE) inhibitor in combination with citrate and thiazide supplementation[80], demonstrating the clinical relevance of a molecular diagnosis for the management of kidney disease.

Another example in which NGS has enabled reclassification of clinical diagnoses is provided by a study in which whole-exome sequencing was performed in 79 consanguineous and familial patients with paediatric onset of CKD with suspect nephronophthisis on the basis of renal ultrasound criteria. A causal mutation was identified in 50 patients, and led to reclassifications of the disease in 18: eight were reclassified as renal tubulopathy, four as Alport syndrome, three as CAKUT, two as autosomal-recessive polycystic kidney disease (OMIM 263200) and one as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome (OMIM 240300)[25]. Here too, reclassification can have important consequences for therapy and clinical monitoring of disease manifestations[85].

Oligogenic inheritance and regulatory regions

Oligogenic inheritance, whereby a phenotype is determined by a combination of mutations in multiple genes, is increasingly recognized to explain at least a component of interfamilial and intrafamilial phenotypic variability. Simultaneous sequencing of genes associated with kidney diseases using NGS has uncovered many genes that contribute modifier alleles to a variety of nephrogenetic diseases, including manifestations outside their original phenotypic spectrum. An example is *HNF1B*, a gene that is involved in a plethora of cystic and noncystic kidney diseases with associated extra-renal manifestations[86]. Heterozygous *HNF1B* mutations have also been detected in combination with mutations in *PKD1* or *PKD2* in familial cases of ADPKD[87]. In these patients, modifier variants in *HNF1B* result in a more severe, early-onset form of ADPKD, which presents antenatally with Potter sequence or enlarged, hyperechoic kidneys. In ciliopathies, heterozygous mutations in *TTC21B*, *RPGRIP1L* and *TMEM67* contribute to mutational load across the ciliopathy spectrum[51,88,89].

Although NGS has boosted gene discovery and significantly improved the diagnostic yield of genetic studies[25,41,90], the origins of many nephrogenetic diseases remain unsolved. The aetiologies of approximately 50% of nephronophthisis cases remain unexplained and genes discovered after the first gene for nephronophthisis, *NPHP1*, in 1997 (REFS 91,92), individually explain the disease in only a very small percentage of patients[93]. Some patients with nephronophthisis have heterozygous mutations in genes associated with recessive disease in which a second mutation remains elusive[94]. These cases warrant new sequencing approaches, such as whole-genome sequencing to study noncoding regulatory regions and microRNAs[90]; chromosome conformation capture[95] or chromatin immunoprecipitation sequencing (in combination with RNA-sequencing or proteomics)[96] to study the regulation of gene expression and epigenetic modifications. An example of a noncoding modifier is an intronic polymorphism in *NOS3* that has been associated with the rate of CKD progression in various populations of patients with ADPKD[97,98]. In a cohort of patients with Alport syndrome, three of 24 identified causal *COL4A5* mutations were intronic[99]. Similarly, whole-genome sequencing in conjunction with mRNA sequencing identified intronic *DGKE* mutations that segregated with aHUS in two independent families[21]. These findings support whole-genome sequencing as a promising approach in genetically unsolved cases[21,100], although the discovery of noncoding mutations brings new challenges in interpreting their functional relevance[101].

Diagnostic opportunities and challenges

Advances in molecular diagnostics could potentially signal the end of the diagnostic renal biopsy. Indeed, molecular diagnostics have many advantages over histopathology, being less invasive and less prone to sampling errors and subjective assessment, which can lead to inconclusive results, especially in advanced stages of disease[102]. In contrast to histopathology, molecular testing requires only venepuncture and has the potential to pinpoint the aetiology of a disease regardless of the disease stage. The advantages of molecular diagnostics are evident in female carriers of Alport syndrome, where the distinction between a heterozygous mutation in the X-linked *COL4A5* gene and a heterozygous mutation in *COL4A3/4* can have important implications for prenatal counselling[75]. In addition, the type of *COL4A* mutation can provide prognostic information regarding renal and extrarenal phenotypes and the risk of post-transplantation anti-GBM glomerulonephritis[75,103], demonstrating that DNA testing can yield a diagnosis and guide prognostic and therapeutic decision-making in patients with nephrogenetic diseases.

Oligogenic inheritance
Inheritance model in which a phenotype is determined by the combination of a few genes.

Chromosome conformation capture
A technique used to study the *in vivo* organization and interactions of genomic elements.

Chromatin immunoprecipitation sequencing
A technique that combines chromatin immunoprecipitation with NGS to study the *in vivo* interaction between proteins (for example transcription factors) or epigenetic modifications (for example histone modifications) and the DNA.

Box 1 | **Current approach for using next-generation sequencing (NGS)-based tests in nephrology**

Requesting genetic tests by non-clinical geneticists

- Nephrologists can order genetic testing of symptomatic patients if the nephrologist is well informed about the inheritance pattern of the likely genes involved and their associated phenotype and has counselled the patient. When gene panels contain large numbers of genes (or the entire exome) with a considerable potential of incidental findings, consider consulting or referring the patient to an expert centre for pre-test counselling.
- Determine the number of genes to be analysed, weighing the chance of finding the causal mutation against the risk of incidental findings.

Pre-test counselling

- Discuss the possibility of finding 'variants of unknown significance' and incidental findings with patients before testing, and explain the hospital's policy with regard to incidental findings. If the implications of an incidental finding in one of the genes in the test are unclear, consult a clinical geneticist.

Post-test counselling

- Correct interpretation of the NGS findings often takes a multidisciplinary approach involving nephrologists, clinical geneticists and laboratory specialists. Consider consulting an expert centre before communicating results to patients.

General considerations

- A diagnosis should be based on a combination of genetic and clinical findings.
- Genetic testing can confirm but not rule out a diagnosis owing to factors such as technical limitations and the presence of unknown causative genes. Some genes (such as *PKD1* and *MUC1*) are notoriously difficult to test. Large copy number variations are often not routinely picked up in diagnostic NGS.
- A genetic diagnosis should be viewed as a starting point that directs further clinical phenotyping.
- If heterozygous mutations in a particular recessive gene can cause a (subtle) phenotype, additional investigations (such as renal ultrasonography or urinalysis) should be considered in carrier parents.
- Oligogenic inheritance should be taken into account to explain interfamilial and intrafamilial variability and in counselling relatives.
- Functional validation, consistent phenotyping and data sharing are essential for the correct interpretation of variants in kidney disease genes.
- A clinical geneticist should be involved in presymptomatic genetic testing of family members.
- Genetic testing of family members, prenatal and preimplantation genetic diagnostics can only be offered if causality of a variant is established. As the total process from mutation to establishing causality can be lengthy, we recommend timely genetic testing.

An obvious limitation of genetic diagnostics, however, is that they cannot be used to diagnose acquired diseases, such as immune-mediated glomerulonephritis. In the absence of evidence for a genetic cause a renal biopsy should therefore be performed if indicated.

One challenge in integrating genetic findings into clinical practice is how to interpret specific genetic variants uncovered by molecular testing. Although many *in silico* tools exist to predict variant pathogenicity, variants that are termed 'pathogenic' in the literature have now also been found in control populations, such as those in the ExAC database, questioning the pathogenicity of these variants. For example, in a patient with ADPKD we observed a p.Arg4154Cys variant in *PKD1* (A.M. van Eerde, unpublished work), which is listed in the Human Gene Mutation Database (HGMD) as a pathogenic mutation on the basis of findings from two publications[104–106]. However, the allele frequency for this variant of 0.001 reported in the ExAC database results in a carrier frequency that is tenfold higher than expected based on the prevalence of ADPKD, the contribution of *PKD1* mutations and the *de novo* mutation rate[107]. Carrier frequencies of this variant in populations of African and South Asian descent are even higher, approaching 1% and 0.6%, respectively. These observations render it highly unlikely that the p.Arg4154Cys variant caused the dominant disease in our patient. Although non-penetrance and variability in age of ADPKD onset have been described, these findings prompt reinterpretation of published 'pathogenic' variants based on their occurrence in control datasets. Genome sequencing is estimated to generate up to 800 putative loss-of-function (LOF) mutations per individual genome (including incorrectly annotated hypomorphic mutations, heterozygous LOF mutations in genes associated with recessive disease and LOF mutations whose effect will be minimal because of gene redundancy), 100 of which are predicted to be true LOF mutations[85]. In a study that investigated LOF mutations in 951 participants between 45 and 65 years of age from the ClinSeq cohort[108], 103 exomes contained putative LOF variants of which 34 could be related to a phenotype through deeper phenotyping or a detailed analysis of family history. The remainder of the LOF mutations, including mutations in *OFD1* (which is typically associated with oral-facial-digital syndrome type 1; OMIM 311200) and *TRPC6* (which is typically associated with FSGS2; OMIM 603965) were also identified in healthy individuals[85], although the possibility that the patient with the *TRPC6* mutation might develop manifestations of

Incidental findings
Findings that are unrelated to the condition for which the DNA test is performed, including alleles that confer disease-risk to the patient as well as carriership for recessive or X-linked disease.

Variants of unknown significance
Variants for which the association with disease risk is unknown.

Copy number variation
A type of structural variation that alters the diploid status of DNA (deletions and duplications).

Loss-of-function
Variant that results in a protein with reduced or no function.

Segregation analysis
Study of the association between a genetic variant and a specific phenotype in a family. It is used to establish the mode of inheritance and to investigate if a specific genetic variant could potentially be causal for the disease in a family.

Canonical
A canonical disease gene is a gene that is typically associated with a particular disease.

Replication stress
Occurs when replication fork progression is slow or problematic and can result in DNA damage and genomic instability.

FSGS later in life cannot be excluded. Similarly, targeted NGS of 208 candidate genes in 453 patients with CAKUT detected no significant excess of rare variants in these genes compared to control cohorts[109]. These findings illustrate that the genetic background of kidney diseases is more complex than previously thought. General considerations with regard to the interpretation of sequence variants in kidney disease and the concomitant ethical implications have been reviewed previously[90].

Another point to bear in mind is that 'pathogenic' mutations are not always causal mutations for the disease of interest. Consequently, molecular testing in an index patient should be followed by segregation analysis in the family to determine whether the findings fit the expected inheritance pattern. If so, further validation of the findings should be performed to obtain clinical and functional proof of involvement of the variant in disease pathogenesis, for example, by performing deeper phenotyping — which can include a revision of the renal biopsy sample and clinical evaluation of renal and extrarenal symptoms — to confirm the genetic diagnosis. Mutations in novel genes or novel mutations (especially missense mutations) in known genes require validation in a larger patient cohort and confirmation of their function, for example in patient-derived cells or model organisms[101]. Of note, the presence of mutations in other genes known to be associated with a particular disease must be excluded before causality can be attributed to a new candidate variant[25,110].

Diverse phenotypes are associated with mutations in canonical kidney disease genes. The examples discussed here show that phenotypic boundaries of disease can shift and that genotype–phenotype correlations are not clear cut[111]. However, the ability to accurately predict the phenotype or outcome of a particular disease is relevant for genetic and reproductive counselling, for instance in decisions regarding preimplantation genetic diagnostics. Consequently, well-maintained international registries that combine extensive genotypic and phenotypic patient data, for example Decipher[112] and ClinVar[113], are imperative to establish clinically useful genotype–phenotype correlations.

Thus, although molecular diagnostics is a valuable addition to the diagnostic toolbox, its diagnostic utility is limited to diseases with a genetic aetiology and the interpretation of genetic variants can be challenging. Genetic counselling and disease management will become increasingly individualized on the basis of specific combinations of clinical findings together with the identification of disease gene mutations, modifiers and epigenetic factors[114,115].

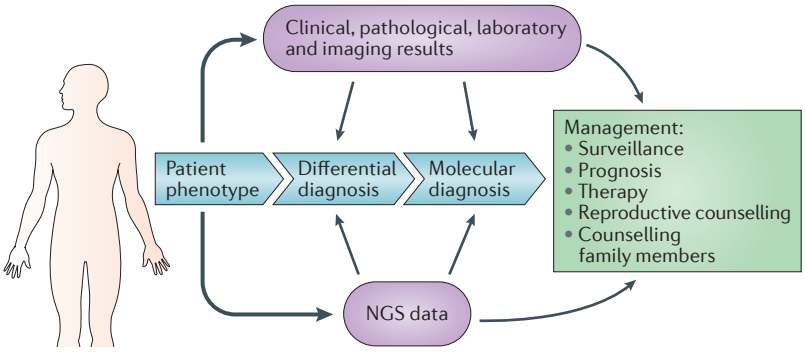

Figure 3 | **Implementation of next-generation sequencing (NGS) data in routine nephrology practice.** When patients enter the nephrology outpatient clinic, NGS will be performed as part of the routine diagnostic workup. NGS results will be considered in combination with the phenotype and additional clinical investigations, including laboratory tests and renal ultrasonography. In the absence of evidence for a genetic cause a renal biopsy can be performed if indicated, for example for suspected immune-mediated glomerulonephritis. Detection of possibly causal genetic variants should be followed by segregation analysis in the family, deeper clinical phenotyping and, in case of novel variants, validation in larger cohorts and/or functional models before a definite molecular diagnosis can be made. NGS data, coupled with clinical data, can be used as a resource to guide and monitor personalized treatments, prognosis and surveillance in patients and family members, and can be used for reproductive counselling.

Personalized treatment of kidney disease

The ultimate goal of studies on the genetic aetiology of kidney phenotypes, alongside genetic counselling, is the development and potential application of personalized approaches to treatment. The identification of mutations in podocyte-specific genes can influence decisions on immunosuppressive treatment in patients with FSGS and inform post-transplantation prognostics[116,117]. Similarly, the post-transplantation recurrence rate of aHUS is much lower in patients with mutations in *MCP* and *DGKE* than in patients with mutations in other genes[42,118] and ACE inhibitors can be used to slow the progression of renal failure in patients with Alport syndrome without the need for immunosuppressants[119,120]. Improved understanding of the genes and mechanisms underlying kidney disease can open new therapeutic routes. For example, the discovery of the role of DNA replication stress in the pathogenesis of nephronophthisis has led to the suggestion that antifibrotic agents such as paclitaxel might represent a promising new therapy for this disease[121]. Despite these successes, the variable response to complement inhibitors in patients with *DGKE*-related nephropathy illustrates that a monogenic paradigm can be too simplistic when considering pharmacogenomics. Large case–control studies are needed to establish real correlations between genetic, epigenetic and environmental factors in relation to response to therapy.

Conclusions

The explosive increase in the availability of high-throughput sequencing techniques has revolutionized the field of nephrogenetics. Large-scale implementation of NGS in research and diagnostics of kidney disease has led to a surprising shift in the phenotypic boundaries of diseases, connecting phenotypes within and across current disease categories and leading to new aetiological insights. For example, the identification of mutations in *DGKE*, a gene that is typically associated with MGPN, in patients with aHUS inspired the investigation of complement-dependent and complement-independent mechanistic links between both

DGKE-associated diseases. The exploration of common aetiologies of diseases will thus continuously improve our understanding of the pathophysiology of inherited kidney disease and expose novel targets for personalized therapies.

An important question is whether molecular diagnostics will eventually replace clinical diagnostic tools. Although genetics can lead to an accurate diagnosis of hereditary kidney diseases, the phenotypic heterogeneity associated with genes described in this Review demonstrates that the aetiology of kidney disease is complex in many cases. We therefore cannot base diagnosis and therapeutic decisions solely on genetic findings (BOX 1). Several mechanisms exist to explain phenotypic heterogeneity, including the type and location of the mutation[54], the presence or absence of basal exon skipping[60], whether oligogenic inheritance is present[51,87], and the influence of noncoding regions and epigenetic regulators[21,96]. While all these factors might influence disease outcome, therapy responsiveness depends on additional genetic factors (such as the presence of cytochrome P450 polymorphisms, which can affect drug metabolism) and environmental factors (such as diet). The foundation of hereditary kidney disease management should therefore be based on molecular genetic analyses closely coupled with clinical evaluation and treatment (FIG. 3). Emerging complex inheritance patterns and new challenges with regard to the interpretation of genetic data require multidisciplinary approaches, innovative computational tools, deeper and consistent phenotyping and international data sharing. A 'genome first' approach, whereby genetic testing is performed at the start of the diagnostic workup and the results integrated into all stages of routine clinical practice, will help to optimize care for patients with kidney disease.

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M.F.S. researched data for the article and wrote the article. All authors made substantial contributions to discussions of the article's content and reviewed/edited the manuscript before submission

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The authors declare no competing interests.

DATABASES
OMIM: http://www.omim.org/
256300 | 173900 | 603278 | 610805 | 256100 | 615008 | 235400 | 301050 | 141200 | 613820 | 613819 | 249000 | 213300 | 194070 | 194080 | 125850 | 616026 | 227810 | 161200 | 309000 | 300555 | 219000 | 601678 | 300009 | 263200 | 240300 | 311200 | 603965
ExAC database: http://exac.broadinstitute.org
Human Gene Mutation Database: www.biobase-international.com/product/hgmd
Decipher: http://decipher.sanger.ac.uk
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