Next-generation sequencing for research and diagnostics in kidney disease

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Abstract | The advent of next-generation sequencing technologies has enabled genetic nephrology research to move beyond single gene analysis to the simultaneous investigation of hundreds of genes and entire pathways. These new sequencing approaches have been used to identify and characterize causal factors that underlie inherited heterogeneous kidney diseases such as nephronophthisis and congenital anomalies of the kidney and urinary tract. In this Review, we describe the development of next-generation sequencing in basic and clinical research and discuss the implementation of this novel technology in routine patient management. Widespread use of targeted and nontargeted approaches for gene identification in clinical practice will require consistent phenotyping, appropriate disease modelling and collaborative efforts to combine and integrate data analyses. Next-generation sequencing is an exceptionally promising technique that has the potential to improve the management of patients with inherited kidney diseases. However, identifying the molecular mechanisms that lead to renal developmental disorders and ciliopathies is difficult. A major challenge in the near future will be how best to integrate data obtained using next-generation sequencing with personalized medicine, including use of high-throughput disease modelling as a tool to support the clinical diagnosis of kidney diseases.

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Introduction

Inherited kidney diseases—the leading cause of chronic kidney disease (CKD) in children—are associated with an increased risk of mortality, cardiovascular morbidity and growth impairment.[1,2] A subset of kidney disorders has been attributed to single gene changes and clear-cut molecular diagnostic tests for these disorders have become available.[3] However, complex inheritance patterns suggest a multigenic aetiology for the majority of inherited kidney diseases. Traditionally, genetic testing in DNA diagnostic laboratories involved sequential Sanger sequencing of known disease genes. However, the diagnostic yield of next-generation sequencing exceeds that of Sanger sequencing in genetically heterogeneous diseases (including inherited kidney disorders) because multiple genes can be analysed in a single experiment.[4] Thus, the introduction of next-generation sequencing has provided revolutionary opportunities for comprehensive genetic testing in research and diagnostics.

Limited knowledge of the genetic background of inherited kidney disorders precludes molecular confirmation of a diagnosis in many patients. Identification of novel disease genes will likely facilitate greater use of molecular diagnostics and reduce the need for invasive diagnostic procedures, such as renal biopsy. Importantly, molecular diagnostics can be performed at an early stage of disease, often enabling a broader set of therapeutic options and a lengthened window of opportunity to ameliorate disease progression.[5] The identification of underlying genetic defects will also improve estimations of recurrence risk during genetic counselling and enable prenatal testing.

Identification of disease genes in nephronophthisis and congenital anomalies of the kidney and urinary tract (CAKUT) is particularly complex because damaging mutations often occur in more than one gene, resulting in digenic or oligogenic inheritance.[3,6,7] This finding suggests that the collective mutational load determines the phenotype of these diseases.[7] Nephronophthisis is generally considered to be a recessive disorder, whereas CAKUT show diverse inheritance patterns, therefore, different filtering strategies apply to the analysis of next-generation sequencing data in these diseases.

In this Review, we outline current use of next-generation sequencing in kidney disease research and diagnostics, focusing on nephronophthisis and CAKUT. We summarize the approaches used for novel gene identification as well as current insight into the genetic background of inherited kidney diseases in the context of technical challenges and ethical considerations. Finally, we highlight the potential of data obtained using next-generation sequencing to improve diagnostics, facilitate prognostics and genetic counselling and enable the development of targeted therapies for patients with inherited kidney diseases.

Advances in sequencing approaches

Sanger sequencing is a straightforward and highly sensitive tool for mutation identification (Box 1).[8–10] However, in genetically heterogeneous disorders with multiple causal genes, sequential Sanger sequencing of all known

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Key points

- Next-generation sequencing has enabled increasingly accurate and cost-effective methods for mutation detection in patients with inherited kidney disorders
- As renal ciliopathies and congenital anomalies of the kidney and urinary tract are highly heterogeneous disorders, high-throughput sequencing approaches are required for identification of causal genes in research and diagnostics
- Challenges for implementation of next-generation sequencing in clinical practice include ethical considerations and accurate interpretation of genetic variants
- National and international multidisciplinary collaborations involving nephrologists, geneticists, biologists and bioinformaticians are crucial to enable elucidation of the genetic backgrounds of inherited kidney diseases in the research and diagnostics settings

and suspected genes is time consuming and not cost-effective.[4] Using next-generation sequencing approaches, complete genomes of individuals can now be determined within days. These methods have been used very successfully to quickly identify causal genetic defects and disease mechanisms.[11]

The appropriate application and combination of novel sequencing methods with conventional gene-discovery strategies should be considered for each patient and research project. For example, single nucleotide polymorphism (SNP) microarray analysis can be used to identify copy number variations and regions of homozygosity in the genome (Box 1).[12] This powerful tool is often used in research and diagnostics to narrow down the genetic region of interest and reduce the number of genes to be analysed. Moreover, the performance of linkage analysis in large families with genetic disorders that have a dominant inheritance pattern can help to identify co-segregating genetic regions for follow-up with next-generation sequencing methods.

Next-generation sequencing is a very versatile technology that is applicable to various research questions. In basic research, samples are sequenced with high coverage (nucleotides are read many times, known as deep sequencing) to detect mutations or large genomic events in a specific subset of cells (for example, populations that occur in low percentages in the kidney). Thus, in basic research, next-generation sequencing enables a small number of cells to be sequenced at a very high resolution. Archived material can also be sequenced in an effort to 'catalogue' genetic variants in small patient cohorts. A large number of genes can be sequenced per sample to detect prognostic and/or predictive genomic biomarkers or novel drug targets (proteins or entire pathways).

The implementation of next-generation sequencing in molecular genetic testing enables a more cost-effective approach than previous methods with a higher diagnostic yield than ever before.[13–15] The most commonly applied approach—whole-exome sequencing—involves targeted capture of protein-coding DNA in combination with massively parallel sequencing, which facilitates determination of all the coding variation present in an individual genome (Box 1). This approach has accelerated the discovery of genes that underlie Mendelian diseases such as nephronophthisis and nephrotic syndrome.[11,16–18] Next-generation sequencing enables thousands of genes to be analysed simultaneously or a smaller subset of genes (a 'mini-genome' or disease-specific panel) to be examined in a single assay. Massively parallel sequencing of a carefully selected part of the genome (for example, the exome or a specific set of genes relevant to a disease phenotype) leads to a higher sequencing coverage than does whole-genome sequencing and, therefore, highly accurate DNA variant calling for the region of interest.[19]

Successful methods for targeted enrichment of pooled multiplexed barcoded samples, such as microarray-based genomic enrichment, molecular inversion probes and in-solution enrichment have been developed to overcome laborious and costly enrichment methods for multiple samples (Box 1).[20–23] These enrichment strategies have made next-generation sequencing feasible in a diagnostic setting. Whole-exome sequencing has been estimated to identify the disease-causing gene in ≥50% of studies that focus on rare, clinically well-defined Mendelian diseases (with a bias towards recessive disorders).[16] The implementation of next-generation sequencing in clinical practice has changed the way genetic counsellors and other clinicians approach genetic testing. For example, comprehensive genetic testing can be applied at an early stage of the diagnostic process, especially when the disease-associated genes are known. These developments set the stage for the application of next-generation sequencing to facilitate clinical diagnosis and personalized disease-risk profiling.

Box 1 | Genetic analysis techniques

Sanger sequencing
This approach (also known as capillary or first-generation sequencing) is based on the incorporation of labelled chain-terminating dideoxynucleotides during polymerase chain reaction (PCR), followed by electrophoretic size separation and subsequent visualization of the label signals.

Single nucleotide polymorphism array
A microarray-based technique used to detect copy number variations (deletions and duplications) in the genome and identify regions of homozygosity. Such regions can serve as a focus for targeted next-generation sequencing in patients with suspected nephrogenetic disorders.

Next-generation sequencing
Next-generation sequencing (also known as massively parallel sequencing) encompasses several high-throughput sequencing approaches.

- Targeted sequencing involves sequencing selected parts of the genome. This approach is especially suitable for the diagnosis of genetically heterogeneous disorders.
- Gene panel sequencing is the simultaneous sequencing of selected known and candidate disease-causing genes.
- Whole-exome sequencing is targeted sequencing of the exome (that is the protein-coding part of the genome), which constitutes 1–2% of the human genome. The application of whole-exome sequencing in diagnostics and research has enabled the discovery of novel disease genes.
- Whole-genome sequencing is sequencing of the entire genome, including non-coding, regulatory DNA.

Enrichment
Enrichment is the selective capture or isolation of regions of interest from a DNA sample prior to sequencing.

DNA pooling
This practical method reduces the costs of sequencing studies that include large numbers of DNA samples. Multiple DNA samples are pooled for genotyping in one experiment. Unique barcoding of each sample is required to enable tracing of individual sample data.

Diagnostics

Sequencing strategies

Previously, Sanger sequencing of one or a few selected genes was the preferred genetic test for patients with inherited kidney diseases. Now, with the advent of next-generation sequencing in genome diagnostics laboratories, the analysis of large gene sets is being applied in kidney disease diagnostics. Two approaches are currently being used; first, whole-exome sequencing followed by analysis of the genes of interest, and second, sequencing of sets of kidney-disease-specific genes. Importantly, the first approach is not hypothesis driven and can identify novel genetic causes of disease. Another advantage is that the experimental set-up is identical for all patients, facilitating quality control. However, whole-exome sequencing is fairly expensive and can generate dozens of variants requiring individual follow-up. The second approach produces high-coverage sequencing data; the costs of targeted sequencing of hundreds of genes at a high coverage are similar to those of Sanger sequencing of one gene per patient.[24] However, gene discovery is not possible and adding new genes to the panel requires redesign of the assay. Currently, both methods are being implemented in genome diagnostics laboratories.

Identifying causal mutations

Next-generation sequencing provides a wealth of data on genetic variation. The use of this approach for novel gene identification in individual patients poses a challenge in determining which variants most likely influence disease.[11] The complexity of data processing, analysis and interpretation requires the development of appropriate bioinformatics tools. Thus, various filtering and prioritization strategies have been employed to determine the causal mutation among variants identified by next-generation sequencing, depending on the presumed mode of inheritance of the disease, pedigree structure, the extent of locus heterogeneity and the combination of outcomes from additional analyses (for example, linkage and copy number variation analyses).[11,25,26] Comparing data generated using next-generation sequencing with in-house and publically available databases and increasing the number of patients and healthy individuals included in analyses have been shown to be useful in gene-hunting strategies for rare Mendelian disorders.[27,28]

A *de novo* mutation is a genetic alteration present in a patient but not in either parent. The *de novo* hypothesis, which states that new mutations might be responsible for dominant diseases in a significant number of sporadic cases,[29–31] might apply to patients with sporadic kidney disease. A *de novo* approach involving exome sequencing in offspring–parent trios (that is, the affected patient and both parents) has been successfully used to identify the causal gene in sporadic cases of various diseases at the extremes of the phenotypic spectrum.[31–34] Such an approach might also be applicable to sporadic patients with kidney disease.

The development and use of consistent, validated bioinformatics pipelines facilitates complex data handling in research and diagnostics. Scoring systems, such as the Residual Variation Intolerance Score (RVIS), can help to identify variants and genes that are most likely to have a role in disease.[35] The RVIS system was developed using data from 6,503 human whole-exome sequences provided by the National Heart, Lung, and Blood Institute Exome Sequencing Project.[36] Genes responsible for Mendelian diseases were shown to be less tolerant to functional genetic variation than genes that do not cause any known disease.[35] Such an intolerance ranking system can aid in interpreting individual genomes and identifying pathogenic mutations for follow-up. A curated method (using a list of causal genes for medically actionable genetic conditions) to classify actionable variants from exome sequencing data has also been described, underscoring the need for a centralized resource that provides information on pathogenicity.[37] Such an approach requires standardized analysis methods, international collaboration for the integration of molecular data, consistent phenotypic data and experimental proof in the appropriate model systems.

Remaining challenges

Interpretation of variants of unknown clinical significance (VUS) remains a major challenge in diagnostics, particularly as resources for functional testing on an individual patient basis are limited. The development of high-throughput models of disease that use patient-derived material might help to clarify the implications of VUS. Furthermore, sharing of data on VUS between institutes is essential to identify overlapping results and determine the importance of these variants. The vast infrastructure and expertise required to implement next-generation sequencing in diagnostics currently make the technique available for only a selection of highly specialized institutions. This situation is likely to change as the cost of next-generation sequencing continues to decrease.

The most important ethical consideration arising from next-generation sequencing technology is whether or not to disclose incidental findings. The frequency of high-penetrance actionable pathogenic or likely pathogenic incidental variants is <3.4% in individuals of European descent and <1.2% in those of African descent.[37] The ethical, legal and counselling issues surrounding the disclosure of genetic variants and data sharing, examining health benefits, patient autonomy and testing in children have been discussed previously[38–41] and are beyond the scope of this Review. However, with regards to incidental findings, there is an increasing consensus that life-saving data and data of immediate clinical utility should be disclosed.[39]

Genetic testing in kidney disease

DNA research and diagnostics in the field of nephrogenetics has particularly benefitted from next-generation sequencing, partly as a result of parallel analyses of the large suite of genes and pathways that regulate renal homeostasis. For example, simultaneous sequencing of 446 candidate genes in 36 children with steroid-resistant nephrotic syndrome (SRNS) revealed definite

or probable pathogenic variants in a subset of 24 SRNS-associated genes in 70% of patients with familial disease and 15% of sporadic patients, demonstrating the potential of this approach in clinical practice.[42] Massive parallel sequencing of the *PKD1* and *PKD2* genes in a cohort of 25 patients with autosomal dominant polycystic kidney disease (ADPKD), showed a sensitivity of 99.2%, with a turnaround time of 1–2 weeks and a 70% reduction in cost compared with Sanger sequencing.[43] These results suggest that next-generation sequencing could replace Sanger sequencing as the standard approach to clinical genetic testing in ADPKD.

In autosomal recessive diseases, such as nephronophthisis, the powerful combination of homozygosity mapping and exome sequencing is a successful strategy to identify causal gene defects.[44] A study that used a method of homozygosity mapping in which SNP-array analysis was used to detect small segments of homozygosity in 72 patients from non-consanguineous families showed that the known causative mutations reside within these segments in 93% of patients with nephronophthisis or SRNS.[45] An unexpected genetic diagnosis of congenital chloride diarrhoea in a patient referred with suspected Bartter syndrome (renal salt-wasting disease) provided proof-of-concept for whole-exome sequencing as a clinical tool in the evaluation of patients with undiagnosed genetic disorders.[46] These studies illustrate the great potential for next-generation sequencing to reduce costs and turnaround times in diagnostics while, most importantly, achieving a detection rate per gene comparable to the Sanger standard.

Targeted capture and sequencing of selected gene panels is a sensitive and cost-effective method for diagnosis of heterogeneous and well-defined syndromes for which multiple causal genes are known, such as Bardet-Biedl Syndrome, nephronophthisis and monogenic diabetes mellitus (also known as maturity-onset diabetes of the young).[13,47] As next-generation sequencing facilitates screening of multiple genes in large patient cohorts, the occurrence of heterogeneity in kidney diseases is less of a challenge when using this approach than with Sanger sequencing. However, pleiotropic effects and complex inheritance patterns with incomplete penetrance remain a challenge. In the future, we anticipate that every patient with newly diagnosed kidney disease will enter a cycle of advanced, next-generation sequencing and renal mini-genome analyses with subsequent diagnosis, therapy and counselling adjustment according to the unique genomic alterations of their specific condition.

Nephronophthisis

Nephronophthisis is one of the renal ciliopathies—a group of inherited kidney disorders characterized by disruption in the function of primary cilia.[48] These antenna-like sensory organelles are present on nearly all interphase nucleated eukaryotic cells, including the apical surface of renal tubular cells.[49] The connection between cilia dysfunction and renal disease was first established by the discovery that polycystin-1, which has a role in ADPKD, is located in the primary cilia.[50,51] The phenotypic spectrum of renal ciliopathies includes ADPKD, autosomal recessive polycystic kidney disease (ARPKD), nephronophthisis, glomerulocystic kidney disease and medullary sponge kidneys.[52]

Nephronophthisis characterized by chronic tubulointerstitial nephritis is the leading genetic cause of end-stage renal disease (ESRD) in children.[53] The estimated incidence is 0.01–0.20 cases per 10,000 live births.[54] The onset of nephronophthisis is usually marked by polydipsia and polyuria as a result of a defect in urine concentration but infantile nephronophthisis might present with severe hypertension.[55] Additional findings include small-to-normal-sized hyperechogenic kidneys with reduced corticomedullary differentiation on abdominal ultrasonography and histopathological alterations characterized by thickened or disrupted tubular basement membranes, tubular atrophy and dilation, interstitial fibrosis and occasional renal cysts.[56–58] In 10–15% of patients, nephronophthisis is associated with extra-renal symptoms, including retinal degeneration (Senior–Løken syndrome, Bardet Biedl syndrome and Alström syndrome), cerebellar vermis hypoplasia (Joubert syndrome), extodermal dysplasia (Sensenbrenner syndrome and Ellis van Creveld syndrome) and skeletal abnormalities (Jeune syndrome).[48]

As symptoms at presentation are nonspecific, nephronophthisis is diagnosed an average of 3.5 years after onset—when kidney damage is at an advanced stage.[59] In addition, a mutation in one of the 17 genes associated with nephronophthisis is detected in only 30–40% of patients, hampering molecular confirmation of diagnosis.[53] The development of next-generation sequencing has contributed to the discovery of novel disease genes in patients with nephronophthisis and will likely accelerate the elucidation of the highly heterogeneous aetiology of this disease.[17,60] Clinical management of the disease should involve a screening plan with accurate phenotyping and detailed clinical history, a thorough clinical examination including screening for extra-renal manifestations, appropriate genetic testing, counselling, specialist referral and regular review.[61]

Genetics

Nephronophthisis is an autosomal recessive disease. Causal mutations in the *NPHP1* gene are detected in up to 30% of patients,[53,62] whereas mutations in the other 16 *NPHP* genes seem to be causal in <3% of patients (Table 1).[62,63]

Next-generation sequencing

The human *NPHP1* to *NPHP9* genes were discovered using linkage analysis and Sanger sequencing approaches. *SDCCAG8* (also known as *NPHP10*) was the first nephronophthisis gene to be identified using a combination of homozygosity mapping and targeted exome sequencing.[17] In this study, in which >800 candidate ciliopathy genes were sequenced, homozygous mutations in *SDCCAG8* were found in 10 affected families.[17] Conversely, *ANKS6* (also known as *NPHP16*) was discovered using targeted sequencing of 32 candidate ciliopathy genes in a cohort of >1,000 patients

Table 1 | Human genes that have been implicated in nephronophthisis

Gene	Alternative name	Location	Gene product	MIM number	Reference(s)
NPHP1	*NPHP1*	2q13	Nephrocystin 1	607100	142, 143
INVS	*NPHP2*	9q31.1	Inversin	243305	144
NPHP3	*NPHP3*	3q22.1	Nephrocystin 3	608002	145
NPHP4	*NPHP4*	1p36.31	Nephrocystin 4	607215	146, 147
IQCB1	*NPHP5*	3q13.33	IQ motif-containing protein B1	609237	148
CEP290	*NPHP6*	12q21.32	Centrosomal protein of 290 kDa	610142	129
GLIS2	*NPHP7*	16p13.3	Zinc finger protein GLIS2	608539	63
RPGRIP1L	*NPHP8*	16q12.2	Retinitis pigmentosa GTPase regulator-interacting protein 1-like	610937	149
NEK8	*NPHP9*	17q11.2	Serine/threonine-protein kinase Nek9	609799	150
SDCCAG8	*NPHP10*	1q43	Serologically defined colon cancer antigen 8	613524	17
TMEM67	*NPHP11*	8q22.1	Meckelin	609884	66
TTC21B	*NPHP12*	2q24.3	Tetratricopeptide repeat protein 21B	612014	151
WDR19	*NPHP13*	4p14	WD repeat-containing protein 19	608151	69
ZNF423	*NPHP14*	16q12.1	Zinc finger protein 423	604557	70
CEP164	*NPHP15*	11q23.2	Centrosomal protein of 164 kDa	614848	70
ANKS6	*NPHP16*	9q22.33	Ankyrin repeat and SAM domain-containing protein 6	615370	60
IFT172	*NPHP17*	2p23.3	Intraflagellar transport protein 172	607386	152

Abbreviation: MIM, Mendelian Inheritance in Man.

with a nephronophthisis-related phenotype; six mutations in six affected families were identified.[60] The latter approach, which enables numerous candidate genes to be sequenced simultaneously in a large patient cohort, might enable the identification and confirmation of many nephronophthisis genes. In a diagnostic setting, targeted sequencing of known nephronophthisis genes identifies causal pathogenic mutations in 12–25% of patients with a nephronophthisis-related ciliopathy.[64–66]

To date, proteomic studies have identified >1,000 different ciliary proteins.[67] Mutations in only 50 genes have been associated with renal ciliopathies and mutations in 17 of these genes have been associated with nephronophthisis.[62] As an estimated 85% of all recessive mutations are located within the coding regions of the genome, whole-exome sequencing is an efficient method for candidate gene discovery.[68] The *WDR19* (*NPHP13*) gene was identified using whole-exome sequencing in a patient with nephronophthisis associated with Sensenbrenner syndrome.[69] Subsequently, *ZNF423* (*NPHP14*) and *CEP164* (*NPHP15*) were identified using homozygosity mapping in conjunction with whole-exome sequencing.[70] A study that identified a causative mutation in seven out of 10 affected brother and sister pairs with a nephronophthisis-related ciliopathy also demonstrated the diagnostic utility of this approach.[68]

Although nephronophthisis typically follows a monogenic inheritance pattern, evidence supports oligogenic inheritance in some patients. An individual who had nephronophthisis as a result of two heterozygous missense mutations in *NPHP2* and *NPHP3* has been described.[7] Furthermore, three mutations in two *NPHP* genes were identified in patients from six different families.[7] A potential epistatic effect of heterozygous *NPHP6* and *AHI1* mutations was suggested in patients with Joubert syndrome and *NPHP1* mutations that contributed to the occurrence of extra-renal symptoms.[6] The occurrence of oligogenic inheritance further emphasizes the necessity of next-generation sequencing approaches in the identification of causative genetic variants for nephronophthisis.

Whole-genome sequencing reveals the complete genetic code of an individual. Some evidence exists for a role of variation in noncoding DNA in ciliopathies. For example, expression levels of two adjacent nonhomologous genes, *TMEM216* and *TMEM138*, were shown to be associated with Joubert syndrome through a mechanism mediated by a regulatory element that binds in a noncoding intergenic DNA region.[71] Mutations in either *TMEM216* or *TMEM138* give rise to an indistinguishable ciliopathy phenotype, suggesting that both proteins have an important role in ciliogenesis.[71,72] Moreover, epigenetic regulation of ciliary Tuba3a protein expression in murine retinal pigment epithelium has been demonstrated using conditional knockdown of DNA methyltransferase 1, which resulted in a defect in photoreceptor outer segment morphogenesis.[73] Thus, noncoding regulatory elements might be important in ciliopathy aetiology. In addition to identification of noncoding regulatory elements, whole-genome sequencing permits detection of unrecognized exonic sequences and structural rearrangements, including copy number variations.[74] As the costs of next-generation sequencing continue to decrease, whole-genome sequencing on a large scale is likely to become more important in the foreseeable future.

Congenital anomalies of the kidney and urinary tract

CAKUT encompasses a range of structural malformations that are a risk factor for early mortality: renal agenesis, kidney dysplasia, multicystic dysplastic kidney, duplex collecting system, obstructive ureteral and urethral abnormalities.[75,76] As environmental factors can be involved in CAKUT aetiology, it is not regarded as a pure genetic (or monogenetic) disorder. CAKUT is the leading cause of ESRD in children and, therefore, has a major impact on growth and maturation, often coupled with disturbed cognitive development.[77,78] As a result of diminished renal function, patients become dependent on dialysis and/or require renal transplantation and have a poor life expectancy. Current genetic testing is incomplete and is usually performed only in syndromic or severe

isolated patients with CAKUT; a conclusive diagnosis is obtained in only a very small percentage of patients.[79] Thus, diagnostics, prognostics and recurrence risk estimation for CAKUT are insufficient. Understanding the causes of CAKUT is of great importance to patients and their families as this will facilitate early detection and enable genetic counselling to be improved.

Genetics

Although familial clustering and the occurrence of multiorgan syndromes with CAKUT phenotypes suggest a role for genetics in disease pathogenesis, the genetic background of human CAKUT is largely unknown.[80–83] As CAKUT is a developmental disorder with a highly heterogeneous background, it is noteworthy that numerous studies in animal models have shown that variants in genes expressed during embryonic kidney development have an important role in disease aetiology.[84,85] Diverse modes of inheritance have been observed for CAKUT, including dominant, recessive, X-linked and complex inheritance patterns.[86–89] To date, the only genetic screening that is offered to patients comprises *HNF1B* and *PAX2* gene mutation analysis. Moreover, most patients with CAKUT are not offered DNA diagnostics.[90]

Previous efforts to identify genetic factors fundamental to human CAKUT mainly focused on genome-wide association studies, linkage analyses and small-scale candidate gene sequencing. This limited approach led to causal gene detection in only a small percentage (≤12%) of the patients investigated.[91] Interestingly, copy number variation was detected in 16.6% of individuals in a large cohort of patients with congenital renal agenesis and hypodysplasia ($n = 522$).[92] These patients had significantly larger gene-disrupting events than matched controls, indicating that large (>100 kb), rare (frequency <0.01) copy number variations in known and novel genes are involved in CAKUT pathogenesis. The most commonly deleted locus was *HNF1B* (in 2.3% of patients).

WNT4, DSTYK, TRAP1, and *TNXB* are novel CAKUT candidate genes.[93–96] Statistically significant associations of *MMP3* genotypes and *MMP3* and *MMP1* haplotypes with CAKUT have also been reported.[97] Novel genomic strategies are necessary to speed up ascertainment of genetic causes for heterogeneous diseases such as CAKUT. Lack of genotype–phenotype correlations and phenotypic heterogeneity also hamper novel gene discovery in this disease. It has been hypothesized that in a substantial proportion of patients with CAKUT, the disease can be explained by rare single gene defects in many different genes.[3] Applying next-generation sequencing in large patient cohorts in combination with the appropriate model systems will make the identification of novel CAKUT genes feasible and enable important advances in DNA diagnostics.

Next-generation sequencing

Efforts to identify genetic abnormalities in renal development have underlined the heterogeneity of CAKUT. Animal mutant models that show disruption of kidney tubulogenesis suggest new candidate genes.[98,99] Mutation screening in large patient groups followed by functional characterization studies are required to investigate whether these candidates genes might cause the disorder in humans. Exome-sequencing experiments have identified variants in *TRAP1* that are likely to cause CAKUT.[94] A study that combined linkage analysis with sequencing of the associated genetic locus detected *DSTYK* gene mutations in CAKUT, suggesting that this gene is a major determinant of human urinary tract development downstream of FGF signalling.[95] Similarly, a deleterious mutation in the *TNXB* gene was identified using coupled linkage and exome analysis in a family with vesicoureteral reflux.[93] Heterozygous mutations in the *FRAS1* and *FREM2* genes have been identified in nonsyndromic CAKUT.[100] Using a whole-exome sequencing approach in families with several fetuses who had bilateral renal agenesis, homozygous mutations in the integrin α8-encoding gene *ITGA8* were identified and functionally characterized.[87] Mutations in 12 of 17 known dominant CAKUT-causing genes were found in a large cohort of 749 individuals from 650 families.[86] Collectively, these results warrant the implementation of next-generation sequencing in diagnostics for CAKUT.

Models of inherited kidney disease

After a few years of preliminary studies, next-generation sequencing remains in an exploratory phase. Screens of thousands of genes in archived patient material and small patient cohorts has resulted in the identification of novel mutated genes in renal diseases; however, the pathophysiological importance of these genes has not yet been comprehensively addressed. An indisputable need exists to functionally test many of the novel variants identified.

To prove that a particular genetic variant identified by any sequencing method actually causes the observed phenotype requires good model systems with measurable outcomes. Example systems frequently used in kidney disease are cellular reporter assays (short-interfering RNA [siRNA], luciferase or green-florescent protein-based assays), electrophysiological assays, *in silico* prediction models, 3D cell culture or tubulogenesis assays, *ex vivo* kidney explant models and a variety of *in vivo* models (Figure 1). 3D spheroid reconstitution assays can be used to investigate the functions of candidate genes in cilia formation. Pathophysiologically relevant murine kidney inner medullary collecting duct cell lines such as IMCD3 or mpkccd are very useful in these assays because they form tubule or spheroid structures within 3 days, making them amenable to siRNA medium-throughput approaches.[101,102] If differences in the frequency, shape and/or length of cilia are observed after siRNA 'knockdown' of a candidate gene, the cells can be reconstituted with the human orthologue (which is not affected by the siRNA designed for the mouse orthologue) to determine whether rescue of the ciliary phenotype occurs. When phenotypic rescue is observed, any human variants found in the candidate gene can be functionally validated. This approach has been extensively validated as a tool for gene discovery in nephronophthisis and other cystic renal diseases such as the Birt–Hogg–Dubé syndrome.[17,70,102–104]

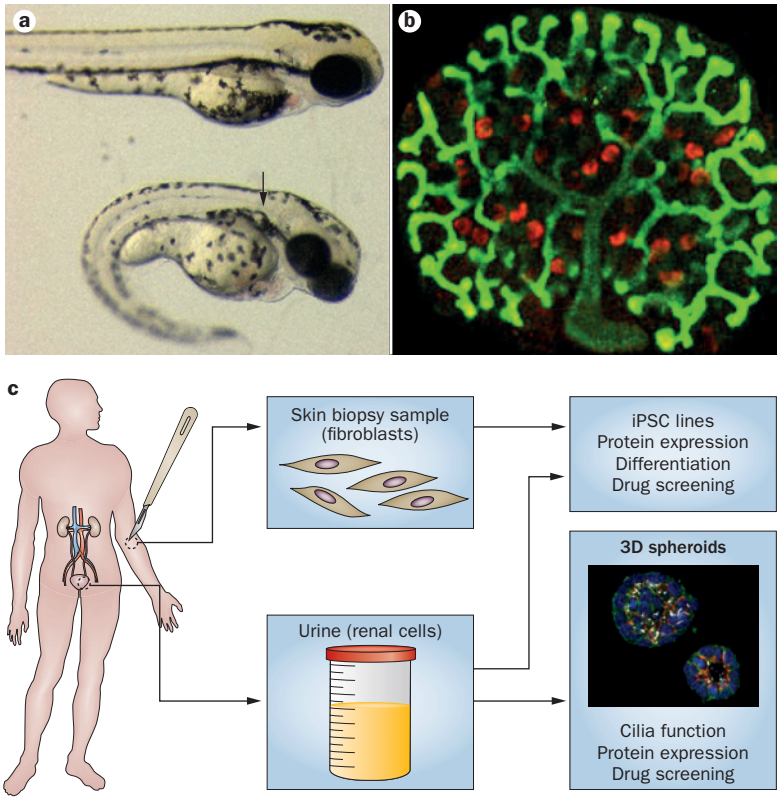

Figure 1 | Kidney disease modelling. **a** | The zebrafish pronephros is a suitable *in vivo* model for renal development and gene expression studies. The function of human variants identified using next-generation sequencing can be tested in this model using gene-silencing and reconstitution assays. The image shows a wild-type zebrafish (top) and a zebrafish with a mutation resulting in a ciliary defect (bottom). The arrow indicates a pronephric cyst. **b** | Mouse kidney explants can be used in developmental studies to investigate the effects of mutations on branching patterns and trace individual cells in the developing kidney. **c** | Fibroblasts or urine cells collected from patients can be used to generate iPSCs, enabling tissue-like and patient-specific *in vitro* studies. Bringing iPSCs into renal differentiation facilitates the identification of patient-specific effects of disturbances in renal cell regulation and cilia function. iPSC lines can also be used to generate 3D renal spheroids in which the effects of candidate gene variants on cilia formation can be studied. Both iPSCs and 3D renal spheroids can be used for high-throughput drug screening. Abbreviaton: iPSC, induced pluripotent stem cell.

Furthermore, modelling nephronophthisis in 3D renal spheroids is tractable to drug intervention studies.[101,103]

The zebrafish pronephros is an appropriate model for testing novel human variants of unknown pathogenicity *in vivo* because it is sensitive to disruptions in ciliary function.[105] As pronephric cysts develop within 4 days of fertilization and can be identified by external examination of the embryo using standard light microscopy, zebrafish have become a favourite model organism in the field. Moreover, microscopic embryos require little space and can be used in high-throughput drug screens (three embryos per well in a 96-well plate).[106] Similar to the 3D spheroid approach, the function of human variants identified using next-generation sequencing can be tested using reconstitution assays. The zebrafish orthologue can be silenced using morpholinos and then reconstituted by co-injection with either the wild-type human orthologue or with patient allelic variants of unknown pathological relevance. For ciliary proteins, a measure of disturbed gastrulation is used as a quantifiable read-out.[107–109]

Modelling CAKUT to test patient variant alleles is somewhat more challenging than modelling nephronophthisis because the underlying molecular defects are diverse and involve several cell types. Cell-based *in vitro* assays will almost certainly not be informative for functional testing of CAKUT patient alleles found using next-generation sequencing unless an individual signalling pathway is to be interrogated (for example Sonic hedgehog signalling).[110] Dissecting the contribution of novel alleles will, therefore, largely rely on tissue-based or *in vivo* assays. For CAKUT, an attractive model that is amenable to siRNA gene knockdown is *ex vivo* renal explants of embryonic mice.[111,112] Whether such assays are useful only for loss-of-function alleles mimicked by siRNA remains to be determined; however, reconstitution of gene knockdown with patient-derived missense alleles seems unlikely to be efficient because levels of transfection and transduction are low throughout entire organs. Renal explants can also be used to trace individual cells in the developing kidney—for example, Lgr5$^+$ stem cells.[113] Combined with genetic manipulation (for example, inducible Cre-based deletion of a gene of interest), lineage tracing could offer a tremendous advance to understanding spatiotemporal factors that influence CAKUT development.

Induced pluripotent stem cells (iPSCs) can also be used to test the function of variants identified using next-generation sequencing. Generating iPSC lines from patient fibroblasts or urine is fairly straightforward and offers the perfect genetic model.[114] Patient-derived iPSCs can be tested for regulated differentiation into relevant renal lineages, accommodating tissue-like studies that can be combined with renal-specific, fluorescently tagged reporter constructs. Stem cell technologies enable the efficient introduction of Bacterial Artificial Chromosomes (BACs) into human iPSC patient and control cell lines.[115] The BAC-reporter approach enables better recapitulation of endogenous gene expression, including microRNA and post-translational regulatory mechanisms, than does standard gene overexpression.[116] Stimulating these lines to undergo renal differentiation will enable patient-specific disturbances in renal cell regulation and/or cilia regulation to be identified. If iPSC lines with an identified gene mutation are used, reconstitution of the wild-type allele (introduced by transcription activator-like effector nuclease-mediated transfer of BAC containing the wild-type gene of interest) can be examined for phenotypic rescue.[117] Initial protocols for differentiation into renal lineages are emerging.[118,119] This approach to examining allelic contribution to disease is particularly exciting and should develop rapidly but it is not yet sufficiently mature to scale up to high-throughput or to be of substantial diagnostic value.

Data integration

Systems biology approaches that involve the integration of molecular datasets to unravel the aetiology of heterogeneous diseases are becoming increasingly feasible.

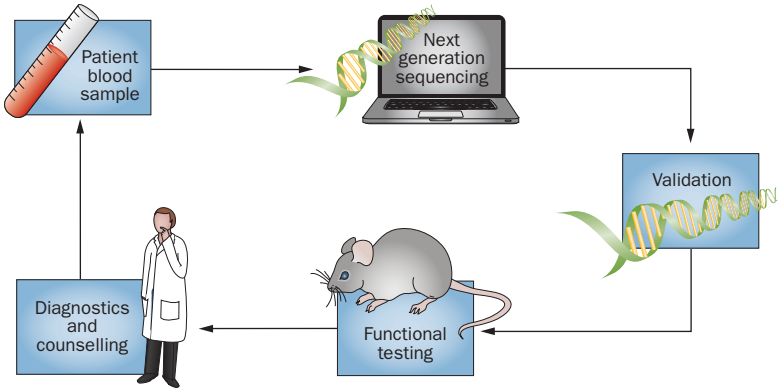

Figure 2 | Next-generation sequencing for the diagnosis of nephrogenetic disorders. Patients with suspected nephrogenetic disorders are referred to nephrologists and clinical geneticists for genetic testing. DNA is isolated from peripheral blood lymphocytes and prepared for next-generation sequencing. A step-wise approach—involving sequencing of a selected gene panel, whole-exome sequencing and whole-genome sequencing in combination with single nucleotide polymorphism array analysis and comparison with the genomes of healthy individuals—is used to identify candidate variants. These variants are validated using Sanger sequencing and segregation analysis in family members. The pathogenicity of variants of unknown clinical significance is determined using functional testing in the appropriate cell or animal models. When the causative mutation is identified, patients and their families receive clinical genetic counselling. Additional genetic testing can be offered to family members.

For example, a combination of tandem affinity purification and mass spectrometry was used to identify seven proteins that interact with the Leber congenital amaurosis-related protein RPGRIP1 and the Joubert and Meckel–Gruber-syndrome-related protein RPGRIP1L (also known as protein fantom).[120] One of these interacting proteins, serine/threonine protein kinase NEK4, was identified as a prominent component of RPGRIP1-associated and RPGRIP1L-associated protein complexes. These complexes are thought to be cilium-specific scaffolds that recruit a NEK4 signalling network, which is important for cilium stability. Downregulation of *NEK4* in ciliated cells led to a substantial decrease in cilium assembly, defining *NEK4* as a ciliopathy candidate gene.[120]

On a larger scale, a similar method was used to identify 850 proteins that interact with nine nephronophthisis, Joubert and Meckel–Gruber syndrome-associated proteins.[102] Analysis of a subset of the corresponding genes identified two novel nephronophthisis and Joubert syndrome genes in 250 patients, *ATXN10* and *TCTN2*. The latter gene was confirmed using a mouse knockout model.[102] These studies demonstrate the efficacy of integrating proteomics data, sequencing data and functional modelling to identify novel disease genes and disease pathways for renal ciliopathies. Furthermore, in a novel reconstitution assay at the proteomic level, reconstitution of a cell line with a missense allele in *LCA5* disrupted the interflagellar interactome, which enabled a molecular signature to be assigned.[121] The combination of chromatin immunoprecipitation followed by sequencing (ChIP-Seq) and RNA sequencing enables the identification of novel transcriptional mechanisms that underlie disease.[122] ChIP-Seq can identify genome-wide transcriptional DNA-binding sites and histone modifications (which regulate gene expression) in intragenic and intergenic regions, whereas RNA sequencing can be used to analyse mRNA levels, splicing variants, noncoding RNA and microRNA on a genome-wide scale.

The generation and integration of datasets from high-throughput experiments requires systematic curation to ensure high-quality data and international comparability. The SYSCILIA consortium aims to assess the quality of high-throughput experiments and to develop bioinformatic and analytical tools to exploit large cilia datasets.[123] An example is the SYSCILIA gold standard: a list of verified ciliary genes that can be used for statistical analyses and as a reference resource.[124] Importantly, integration of various datasets and the connection to human disease requires accurate and consistent phenotyping. Genotypic and phenotypic heterogeneity hampers the accurate assessment of clinical manifestations, leading to an erroneous or ambiguous clinical diagnosis.[125] The Human Phenotype Ontology is emerging as a widely accepted standardized phenotyping methodology to facilitate diagnostics and correlation analyses.[126,127]

Novel therapeutic targets

A major task in inherited kidney disease research is the development of noninvasive therapies to prevent disease progression towards ESRD. Currently, the treatment options for renal ciliopathies are limited to the correction of fluid and electrolyte imbalances, dialysis and renal transplantation. Next-generation sequencing has enabled greater understanding of the pathogenesis of disease; progress in the treatment of genetic disorders is the next step. Systems biology approaches that integrate data from different sources can be applied to identify novel therapeutic targets and model organisms can then be used to screen potential drugs in an unbiased approach. Moreover, investigating the pharmacodynamics of effective drugs identified using a drug screen might expose underlying molecular mechanisms.

Several examples exist of use of model organisms to test potential drug targets. An early report describes use of the PCK rat model of ARPKD and the *pcy* murine model of nephronophthisis to determine the efficacy of a vasopressin V2 receptor antagonist in ameliorating the kidney phenotypes.[128] Administration of this agent resulted in a reduction in renal volume and renal cyst formation, presumably through lowering of renal cAMP levels. The role of cAMP in ciliopathies is unknown but the protein encoded by *NPHP6* (CEP290) modulates activity of the transcription factor ATF4, which is implicated in cAMP-dependent cystogenesis.[129,130] Various signalling pathways implicated in ciliopathies can be targeted with medication. For example, inhibition of the mammalian target of rapamycin (mTOR) pathway, which is regulated by polycystin-1, has been used successfully to treat PKD in animal models.[131,132] In zebrafish models of renal ciliopathies (generated using morpholino oligonucleotides), addition of the mTOR inhibitor rapamycin rescued the phenotype of renal cysts and severe oedema and restored renal function.[133] Similar results were obtained using the cyclin-dependent kinase inhibitor roscovitine. In an

untargeted approach, screening of a library of 115 well-defined chemical compounds targeted at various signalling pathways in zebrafish models of PKD identified histone deacetylase inhibitors as compounds that moderate body curvature, defective laterality and kidney cyst formation.[134] The researchers speculate that these inhibitors suppress the process of cell proliferation that induces cyst formation in patients with PKD.

Future treatments for nephrogenetic disorders comprise gene therapies and regenerative medicine. The potential of gene therapy has been demonstrated in syndromic ciliopathies, such as Bardet–Biedl syndrome. Ectopic expression of the human *BBS4* gene rescued retinal, obese and hydrocephalus phenotypes in *Bbs4*-deficient mice despite tissue-specific variable expression of the transgene.[135] Adult zebrafish can generate new nephrons in response to kidney injury but this phenomenon has not been observed in mammals. The identification of nephron progenitor cells, as well as the genes and molecular pathways that underlie neonephrogenesis in zebrafish offers a promising prospective for renal regeneration in humans.[136]

Conclusions

Understanding the genetic background of inherited kidney diseases is essential to improve the clinical care of affected patients. Next-generation sequencing has contributed to the discovery of novel disease genes and greatly improved the diagnostics toolbox for inherited kidney disease. This technology will further accelerate the elucidation of the highly heterogeneous aetiology of nephronophthisis and CAKUT. Variable disease outcomes in patients with the same genetic defect suggest an important role for modifiers in disease aetiology and progression.[137–139] This role should be investigated as these modifiers might have an important role in CAKUT and nephronophthisis aetiology.

In Europe, large ongoing collaborative efforts including the EURenOmics and SYSCILIA consortia facilitate the integrated use of large well-phenotyped cohorts, the generation of translational patient databases, application of next-generation sequencing platforms and use of various disease models that enable functional testing of patient variants of unknown pathogenicity.[123,140] For rare diseases with complex genetics and heterogeneous phenotypes, these types of multinational consortia form optimal infrastructure to reach mutual goals and directly implement findings into clinical practice. Furthermore, data collection can be improved by giving patients an active role in the form of online questionnaires at multiple time points. This approach facilitates the collection of a wealth of unique information that would be difficult to obtain in the clinic to a similar extent.[141]

Critical to the success of next-generation sequencing in nephrology clinical practice is the awareness of nephrologists (particularly paediatric nephrologists) and clinical geneticists (the phenotyping experts and subsequently the applicants for the appropriate genetic test). Multidisciplinary outpatient clinics provide a centre of expertise and facilitate a very efficient collaborative approach that stimulates the use of next-generation sequencing in general clinical practice (Figure 2). Such collaborations and the implementation of new sequencing technologies are likely to enable optimal patient care.

Review criteria

A search for full-text original research and review articles on the genetics of inherited kidney diseases, focusing on congenital abnormalities of the kidney and urinary tract (CAKUT) and nephronophthisis, was performed in PubMed. Search terms used included “next-generation sequencing”, “kidney disease”, “nephronophthisis” and “CAKUT”, alone and in combination. We also searched the reference lists of identified articles for further relevant papers.

1. Mong Hiep, T. T. *et al.* Clinical characteristics and outcomes of children with stage 3–5 chronic kidney disease. *Pediatr. Nephrol.* **25**, 935–940 (2010).
2. Greenbaum, L. A., Warady, B. A. & Furth, S. L. Current advances in chronic kidney disease in children: growth, cardiovascular, and neurocognitive risk factors. *Semin. Nephrol.* **29**, 425–434 (2009).
3. Hildebrandt, F. Genetic kidney diseases. *Lancet* **375**, 1287–1295 (2010).
4. Neveling, K. *et al.* A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. *Hum. Mutat.* **34**, 1721–1726 (2013).
5. Pei, Y. & Watnick, T. Diagnosis and screening of autosomal dominant polycystic kidney disease. *Adv. Chronic Kidney Dis.* **17**, 140–152 (2010).
6. Tory, K. *et al.* High NPHP1 and NPHP6 mutation rate in patients with Joubert syndrome and nephronophthisis: potential epistatic effect of *NPHP6* and *AHI1* mutations in patients with *NPHP1* mutations. *J. Am. Soc. Nephrol.* **18**, 1566–1575 (2007).
7. Hoefele, J. *et al.* Evidence of oligogenic inheritance in nephronophthisis. *J. Am. Soc. Nephrol.* **18**, 2789–2795 (2007).
8. Sanger, F. & Coulson, A. R. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol.* **94**, 441–448 (1975).
9. Sanger, F., Nicklen, S. & Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl Acad. Sci. USA* **74**, 5463–5467 (1977).
10. Thomas, R. *et al.* Identification of mutations in the repeated part of the autosomal dominant polycystic kidney disease type 1 gene, PKD1, by long-range PCR. *Am. J. Hum. Genet.* **65**, 39–49 (1999).
11. Bamshad, M. J. *et al.* Exome sequencing as a tool for Mendelian disease gene discovery. *Nat. Rev. Genet.* **12**, 745–755 (2011).
12. Hehir-Kwa, J. Y., Pfundt, R., Veltman, J. A. & de Leeuw, N. Pathogenic or not? Assessing the clinical relevance of copy number variants. *Clin. Genet.* **84**, 415–421 (2013).
13. Drmanac, R. The advent of personal genome sequencing. *Genet. Med.* **13**, 188–190 (2011).
14. Need, A. C. *et al.* Clinical application of exome sequencing in undiagnosed genetic conditions. *J. Med. Genet.* **49**, 353–361 (2012).
15. Yang, Y. *et al.* Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N. Engl. J. Med.* **369**, 1502–1511 (2013).
16. Gilissen, C., Hoischen, A., Brunner, H. G. & Veltman, J. A. Unlocking Mendelian disease using exome sequencing. *Genome Biol.* **12**, 228 (2011).
17. Otto, E. A. *et al.* Candidate exome capture identifies mutation of *SDCCAG8* as the cause of a retinal-renal ciliopathy. *Nat. Genet.* **42**, 840–850 (2010).
18. Lovric, S. *et al.* Rapid detection of monogenic causes of childhood-onset steroid-resistant nephrotic syndrome. *Clin. J. Am. Soc. Nephrol.* http://dx.doi.org/10.2215/CJN.09010813.
19. Nijman, I. J. *et al.* Mutation discovery by targeted genomic enrichment of multiplexed barcoded samples. *Nat. Methods* **7**, 913–915 (2010).
20. Cummings, N. *et al.* Combining target enrichment with barcode multiplexing for high throughput SNP discovery. *BMC Genomics* **11**, 641 (2010).
21. Harakalova, M. *et al.* Multiplexed array-based and in-solution genomic enrichment for flexible and cost-effective targeted next-generation sequencing. *Nat. Protoc.* **6**, 1870–1886 (2011).
22. Teer, J. K. *et al.* Systematic comparison of three genomic enrichment methods for massively parallel DNA sequencing. *Genome Res.* **20**, 1420–1431 (2010).

23. Turner, E. H., Ng, S. B., Nickerson, D. A. & Shendure, J. Methods for genomic partitioning. *Annu. Rev. Genomics Hum. Genet.* **10**, 263–284 (2009).
24. Frank, M., Prenzler, A., Eils, R. & Graf von der Schulenburg, J. M. Genome sequencing: a systematic review of health economic evidence. *Health Econ. Rev.* **3**, 29 (2013).
25. Fuchs, S. A. *et al.* Application of exome sequencing in the search for genetic causes of rare disorders of copper metabolism. *Metallomics* **4**, 606–613 (2012).
26. Gilissen, C., Hoischen, A., Brunner, H. G. & Veltman, J. A. Disease gene identification strategies for exome sequencing. *Eur. J. Hum. Genet.* **20**, 490–497 (2012).
27. Zhang, X. Exome sequencing greatly expedites the progressive research of Mendelian diseases. *Front. Med.* **8**, 42–57 (2014).
28. International HapMap 3 Consortium *et al.* Integrating common and rare genetic variation in diverse human populations. *Nature* **467**, 52–58 (2010).
29. Crow, J. F. The origins, patterns and implications of human spontaneous mutation. *Nat. Rev. Genet.* **1**, 40–47 (2000).
30. Jouan, L., Gauthier, J., Dion, P. A. & Rouleau, G. A. Rare variants in complex traits: novel identification strategies and the role of *de novo* mutations. *Hum. Hered.* **74**, 215–225 (2012).
31. Veltman, J. A. & Brunner, H. G. *De novo* mutations in human genetic disease. *Nat. Rev. Genet.* **13**, 565–575 (2012).
32. de Ligt, J. *et al.* Diagnostic exome sequencing in persons with severe intellectual disability. *N. Engl. J. Med.* **367**, 1921–1929 (2012).
33. Vissers, L. E. *et al.* A *de novo* paradigm for mental retardation. *Nat. Genet.* **42**, 1109–1112 (2010).
34. Xu, B. *et al. De novo* gene mutations highlight patterns of genetic and neural complexity in schizophrenia. *Nat. Genet.* **44**, 1365–1369 (2012).
35. Petrovski, S., Wang, Q., Heinzen, E. L., Allen, A. S. & Goldstein, D. B. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet.* **9**, e1003709 (2013).
36. NHLBI Exome Sequencing Project. Exome Variant Server. *evs.gs.washington.edu* [online], http://evs.gs.washington.edu/EVS (2014).
37. Dorschner, M. O. *et al.* Actionable, pathogenic incidental findings in 1,000 participants' exomes. *Am. J. Hum. Genet.* **93**, 631–640 (2013).
38. Abdul-Karim, R. *et al.* Disclosure of incidental findings from next-generation sequencing in pediatric genomic research. *Pediatrics* **131**, 564–571 (2013).
39. Christenhusz, G. M., Devriendt, K. & Dierickx, K. To tell or not to tell? A systematic review of ethical reflections on incidental findings arising in genetics contexts. *Eur. J. Hum. Genet.* **21**, 248–255 (2013).
40. Lolkema, M. P. *et al.* Ethical, legal, and counseling challenges surrounding the return of genetic results in oncology. *J. Clin. Oncol.* **31**, 1842–1848 (2013).
41. Bredenoord, A. L., Kroes, H. Y., Cuppen, E., Parker, M. & van Delden, J. J. Disclosure of individual genetic data to research participants: the debate reconsidered. *Trends Genet.* **27**, 41–47 (2011).
42. McCarthy, H. J. *et al.* Simultaneous sequencing of 24 genes associated with steroid-resistant nephrotic syndrome. *Clin. J. Am. Soc. Nephrol.* **8**, 637–648 (2013).
43. Tan, A. Y. *et al.* molecular diagnosis of autosomal dominant polycystic kidney disease using next-generation sequencing. *J. Mol. Diagn.* **16**, 216–228 (2013).
44. Below, J. E. *et al.* Whole-genome analysis reveals that mutations in inositol polyphosphate phosphatase-like 1 cause opsismodysplasia. *Am. J. Hum. Genet.* **92**, 137–143 (2013).
45. Hildebrandt, F. *et al.* A systematic approach to mapping recessive disease genes in individuals from outbred populations. *PLoS Genet.* **5**, e1000353 (2009).
46. Choi, M. *et al.* Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc. Natl Acad. Sci. USA* **106**, 19096–19101 (2009).
47. Katsanis, S. H. & Katsanis, N. Molecular genetic testing and the future of clinical genomics. *Nat. Rev. Genet.* **14**, 415–426 (2013).
48. Hildebrandt, F., Benzing, T. & Katsanis, N. Ciliopathies. *N. Engl. J. Med.* **364**, 1533–1543 (2011).
49. Eggenschwiler, J. T. & Anderson, K. V. Cilia and developmental signaling. *Annu. Rev. Cell Dev. Biol.* **23**, 345–373 (2007).
50. Barr, M. M. & Sternberg, P. W. A polycystic kidney-disease gene homologue required for male mating behaviour in, *C. elegans*. *Nature* **401**, 386–389 (1999).
51. The European Polycystic Kidney Disease Consortium. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell* **77**, 881–894 (1994).
52. Gunay-Aygun, M. Liver and kidney disease in ciliopathies. *Am. J. Med. Genet. C Semin. Med. Genet.* **151C**, 296–306 (2009).
53. Hurd, T. W. & Hildebrandt, F. Mechanisms of nephronophthisis and related ciliopathies. *Nephron Exp. Nephrol.* **118**, e9–e14 (2011).
54. Hildebrandt, F., Attanasio, M. & Otto, E. Nephronophthisis: disease mechanisms of a ciliopathy. *J. Am. Soc. Nephrol.* **20**, 23–35 (2009).
55. Gagnadoux, M. F., Bacri, J. L., Broyer, M. & Habib, R. Infantile chronic tubulo-interstitial nephritis with cortical microcysts: variant of nephronophthisis or new disease entity? *Pediatr. Nephrol.* **3**, 50–55 (1989).
56. Blowey, D. L., Querfeld, U., Geary, D., Warady, B. A. & Alon, U. Ultrasound findings in juvenile nephronophthisis. *Pediatr. Nephrol.* **10**, 22–24 (1996).
57. Chung, E. M., Conran, R. M., Schroeder, J. W., Rohena-Quinquilla, I. R. & Rooks, V. J. From the radiologic pathology archives: pediatric polycystic kidney disease and other ciliopathies: radiologic-pathologic correlation. *Radiographics* **34**, 155–178 (2014).
58. Waldherr, R., Lennert, T., Weber, H. P., Fodisch, H. J. & Scharer, K. The nephronophthisis complex. A clinicopathologic study in children. *Virchows Arch. A Pathol. Anat. Histol.* **394**, 235–254 (1982).
59. Soliman, N. A. *et al.* Clinical characterization and NPHP1 mutations in nephronophthisis and associated ciliopathies: a single center experience. *Saudi J. Kidney Dis. Transpl.* **23**, 1090–1098 (2012).
60. Hoff, S. *et al.* ANKS6 is a central component of a nephronophthisis module linking NEK8 to INVS and NPHP3. *Nat. Genet.* **45**, 951–956 (2013).
61. Simms, R. J., Hynes, A. M., Eley, L. & Sayer, J. A. Nephronophthisis: a genetically diverse ciliopathy. *Int. J. Nephrol.* **2011**, 527137 (2011).
62. Arts, H. H. & Knoers, N. V. Current insights into renal ciliopathies: what can genetics teach us? *Pediatr. Nephrol.* **28**, 863–874 (2013).
63. Attanasio, M. *et al.* Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. *Nat. Genet.* **39**, 1018–1024 (2007).
64. Halbritter, J. *et al.* High-throughput mutation analysis in patients with a nephronophthisis-associated ciliopathy applying multiplexed barcoded array-based PCR amplification and next-generation sequencing. *J. Med. Genet.* **49**, 756–767 (2012).
65. Halbritter, J. *et al.* Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy. *Hum. Genet.* **132**, 865–884 (2013).
66. Otto, E. A. *et al.* Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy. *J. Med. Genet.* **48**, 105–116 (2011).
67. Yuan, S. & Sun, Z. Expanding horizons: ciliary proteins reach beyond cilia. *Annu. Rev. Genet.* **47**, 353–376 (2013).
68. Gee, H. Y. *et al.* Whole-exome resequencing distinguishes cystic kidney diseases from phenocopies in renal ciliopathies. *Kidney Int.* (2013).
69. Bredrup, C. *et al.* Ciliopathies with skeletal anomalies and renal insufficiency due to mutations in the IFT-A gene WDR19. *Am. J. Hum. Genet.* **89**, 634–643 (2011).
70. Chaki, M. *et al.* Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. *Cell* **150**, 533–548 (2012).
71. Lee, J. H. *et al.* Evolutionarily assembled cis-regulatory module at a human ciliopathy locus. *Science* **335**, 966–969 (2012).
72. Edvardson, S. *et al.* Joubert syndrome 2 (JBTS2) in Ashkenazi Jews is associated with a *TMEM216* mutation. *Am. J. Hum. Genet.* **86**, 93–97 (2010).
73. Nasonkin, I. O. *et al.* Conditional knockdown of DNA methyltransferase 1 reveals a key role of retinal pigment epithelium integrity in photoreceptor outer segment morphogenesis. *Development* **140**, 1330–1341 (2013).
74. Nishiguchi, K. M. *et al.* Whole genome sequencing in patients with retinitis pigmentosa reveals pathogenic DNA structural changes and NEK2 as a new disease gene. *Proc. Natl Acad. Sci. USA* **110**, 16139–16144 (2013).
75. Loane, M. *et al.* Paper 4: EUROCAT statistical monitoring: identification and investigation of ten year trends of congenital anomalies in Europe. *Birth Defects Res. A Clin. Mol. Teratol.* **91** (Suppl. 1), S31–S43 (2011).
76. Melo, B. F. *et al.* Early risk factors for neonatal mortality in CAKUT: analysis of 524 affected newborns. *Pediatr. Nephrol.* **27**, 965–972 (2012).
77. Groothoff, J. W. *et al.* Social consequences in adult life of end-stage renal disease in childhood. *J. Pediatr.* **146**, 512–517 (2005).
78. European Renal Association–European dialysis and Transplant Association. ERA–EDTA Registry Annual Report 2011. *era-edta-reg.org* [online], http://www.era-edta-reg.org/files/annualreports/pdf/AnnRep2011.pdf (2011).
79. Madariaga, L. *et al.* Severe prenatal renal anomalies associated with mutations in *HNF1B* or *PAX2* genes. *Clin. J. Am. Soc. Nephrol.* **8**, 1179–1187 (2013).
80. Belk, R. A. *et al.* A family study and the natural history of prenatally detected unilateral multicystic dysplastic kidney. *J. Urol.* **167**, 666–669 (2002).
81. Bulum, B. *et al.* High frequency of kidney and urinary tract anomalies in asymptomatic first-degree relatives of patients with CAKUT. *Pediatr. Nephrol.* **28**, 2143–2147 (2013).

82. Horikawa, Y. *et al.* Mutation in hepatocyte nuclear factor-1β gene (TCF2) associated with MODY. *Nat. Genet.* **17**, 384–385 (1997).
83. Winyard, P. & Chitty, L. S. Dysplastic kidneys. *Semin. Fetal Neonatal Med.* **13**, 142–151 (2008).
84. Little, M., Georgas, K., Pennisi, D. & Wilkinson, L. Kidney development: two tales of tubulogenesis. *Curr. Top. Dev. Biol.* **90**, 193–229 (2010).
85. Schedl, A. Renal abnormalities and their developmental origin. *Nat. Rev. Genet.* **8**, 791–802 (2007).
86. Hwang, D. Y. *et al.* Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract. *Kidney Int.* http://dx.doi.org/10.1038/ki.2013.508.
87. Humbert, C. *et al.* Integrin α8 recessive mutations are responsible for bilateral renal agenesis in humans. *Am. J. Hum. Genet.* **94**, 288–294 (2014).
88. Naseri, M., Ghiggeri, G. M., Caridi, G. & Abbaszadegan, M. R. Five cases of severe vesico-ureteric reflux in a family with an X-linked compatible trait. *Pediatr. Nephrol.* **25**, 349–352 (2010).
89. Kerecuk, L. *et al.* Autosomal dominant inheritance of non-syndromic renal hypoplasia and dysplasia: dramatic variation in clinical severity in a single kindred. *Nephrol. Dial. Transplant.* **22**, 259–263 (2007).
90. Fletcher, J., McDonald, S., Alexander, S. I. & Australian & New Zealand Pediatric Nephrology Association. Prevalence of genetic renal disease in children. *Pediatr. Nephrol.* **28**, 251–256 (2013).
91. Renkema, K. Y. *et al.* Novel perspectives for investigating congenital anomalies of the kidney and urinary tract (CAKUT). *Nephrol. Dial. Transplant*. **26**, 3843–3851 (2011).
92. Sanna-Cherchi, S. *et al.* Copy-number disorders are a common cause of congenital kidney malformations. *Am. J. Hum. Genet.* **91**, 987–997 (2012).
93. Gbadegesin, R. A. *et al. TNXB* mutations can cause vesicoureteral reflux. *J. Am. Soc. Nephrol.* **24**, 1313–1322 (2013).
94. Saisawat, P. *et al.* Whole-exome resequencing reveals recessive mutations in TRAP1 in individuals with CAKUT and VACTERL association. *Kidney Int.* http://dx.doi.org/10.1038/ki.2013.417.
95. Sanna-Cherchi, S. *et al.* Mutations in DSTYK and dominant urinary tract malformations. *N. Engl. J. Med.* **369**, 621–629 (2013).
96. Vivante, A. *et al.* Renal hypodysplasia associates with a WNT4 variant that causes aberrant canonical WNT signaling. *J. Am. Soc. Nephrol.* **24**, 550–558 (2013).
97. Djuric, T. *et al.* MMP-1 and -3 haplotype is associated with congenital anomalies of the kidney and urinary tract. *Pediatr. Nephrol.* **29**, 879–884 (2014).
98. Barak, H. *et al.* FGF9 and FGF20 maintain the stemness of nephron progenitors in mice and man. *Dev. Cell* **22**, 1191–1207 (2012).
99. Kaku, Y. *et al.* Islet1 deletion causes kidney agenesis and hydroureter resembling CAKUT. *J. Am. Soc. Nephrol.* **24**, 1242–1249 (2013).
100. Saisawat, P. *et al.* Identification of two novel CAKUT-causing genes by massively parallel exon resequencing of candidate genes in patients with unilateral renal agenesis. *Kidney Int.* **81**, 196–200 (2012).
101. de Groot, T. *et al.* Lithium causes G2 arrest of renal principal cells. *J. Am. Soc. Nephrol.* **25**, 501–510 (2014).
102. Sang, L. *et al.* Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. *Cell* **145**, 513–528 (2011).
103. Choi, H. J. *et al.* NEK8 links the ATR-regulated replication stress response and S phase CDK activity to renal ciliopathies. *Mol. Cell* **51**, 423–439 (2013).
104. Luijten, M. N. *et al.* Birt-Hogg-Dube syndrome is a novel ciliopathy. *Hum. Mol. Genet.* **22**, 4383–4397 (2013).
105. Ebarasi, L., Oddsson, A., Hultenby, K., Betsholtz, C. & Tryggvason, K. Zebrafish: a model system for the study of vertebrate renal development, function, and pathophysiology. *Curr. Opin. Nephrol. Hypertens.* **20**, 416–424 (2011).
106. Kaufman, C. K., White, R. M. & Zon, L. Chemical genetic screening in the zebrafish embryo. *Nat. Protoc.* **4**, 1422–1432 (2009).
107. Basten, S. G. *et al.* Mutations in LRRC50 predispose zebrafish and humans to seminomas. *PLoS Genet.* **9**, e1003384 (2013).
108. Ross, A. J. *et al.* Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat. Genet.* **37**, 1135–1140 (2005).
109. Zaghloul, N. A. & Katsanis, N. Zebrafish assays of ciliopathies. *Methods Cell Biol.* **105**, 257–272 (2011).
110. Feeney, M. M. & Rosenblum, N. D. Urinary tract pacemaker cells: current knowledge and insights from nonrenal pacemaker cells provide a basis for future discovery. *Pediatr. Nephrol.* **4**, 629–635 (2013).
111. Burn, S. F. *et al.* Calcium/NFAT signalling promotes early nephrogenesis. *Dev. Biol.* **352**, 288–298 (2011).
112. Davies, J. A. *et al.* Development of an siRNA-based method for repressing specific genes in renal organ culture and its use to show that the Wt1 tumour suppressor is required for nephron differentiation. *Hum. Mol. Genet.* **13**, 235–246 (2004).
113. Barker, N. *et al.* $Lgr5^{+ve}$ stem/progenitor cells contribute to nephron formation during kidney development. *Cell Rep.* **2**, 540–552 (2012).
114. Inoue, H., Nagata, N., Kurokawa, H. & Yamanaka, S. iPS cells: a game changer for future medicine. *EMBO J.* **33**, 409–417 (2014).
115. Woltjen, K. *et al.* piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* **458**, 766–770 (2009).
116. Meir, Y. J. *et al.* A versatile, highly efficient, and potentially safer piggyBac transposon system for mammalian genome manipulations. *FASEB J.* **27**, 4429–4443 (2013).
117. Hockemeyer, D. *et al.* Genetic engineering of human pluripotent cells using TALE nucleases. *Nat. Biotechnol.* **29**, 731–734 (2011).
118. Lam, A. Q. *et al.* Rapid and efficient differentiation of human pluripotent stem cells into intermediate mesoderm that forms tubules expressing kidney proximal tubular markers. *J. Am. Soc. Nephrol.* http://dx.doi.org/10.1681/ASN.2013080831.
119. Takasato, M. *et al.* Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat. Cell Biol.* **16**, 118–126 (2014).
120. Coene, K. L. *et al.* The ciliopathy-associated protein homologs RPGRIP1 and RPGRIP1L are linked to cilium integrity through interaction with Nek4 serine/threonine kinase. *Hum. Mol. Genet.* **20**, 3592–3605 (2011).
121. Boldt, K. *et al.* Disruption of intraflagellar protein transport in photoreceptor cilia causes Leber congenital amaurosis in humans and mice. *J. Clin. Invest.* **121**, 2169–2180 (2011).
122. Mimura, I., Kanki, Y., Kodama, T. & Nangaku, M. Revolution of nephrology research by deep sequencing: ChIP-seq and RNA-seq. *Kidney Int.* **85**, 31–38 (2014).
123. European project SYSCILIA. *Syscilia* [online], http://www.syscilia.org/index.shtml (2014).
124. van Dam, T. J. *et al.* The SYSCILIA gold standard (SCGSv1) of known ciliary components and its applications within a systems biology consortium. *Cilia* **2**, 7 (2013).
125. Ku, C. S. *et al.* Exome sequencing: dual role as a discovery and diagnostic tool. *Ann. Neurol.* **71**, 5–14 (2012).
126. Robinson, P. N. *et al.* The Human Phenotype Ontology: a tool for annotating and analyzing human hereditary disease. *Am. J. Hum. Genet.* **83**, 610–615 (2008).
127. The Human Phenotype Ontology Project. *Human Phenotype Ontology Website* [online], www.human-phenotype-ontology.org/ (2014).
128. Gattone, V. H. 2nd, Wang, X., Harris, P. C. & Torres, V. E. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat. Med.* **9**, 1323–1326 (2003).
129. Sayer, J. A. *et al.* The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat. Genet.* **38**, 674–681 (2006).
130. Torres, V. E. *et al.* Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat. Med.* **10**, 363–364 (2004).
131. Shillingford, J. M. *et al.* The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc. Natl Acad. Sci. USA* **103**, 5466–5471 (2006).
132. Wang, S. & Dong, Z. Primary cilia and kidney injury: current research status and future perspectives. *Am. J. Physiol. Renal Physiol.* **305**, F1085–F1098 (2013).
133. Tobin, J. L. & Beales, P. L. Restoration of renal function in zebrafish models of ciliopathies. *Pediatr. Nephrol.* **23**, 2095–2099 (2008).
134. Cao, Y. *et al.* Chemical modifier screen identifies HDAC inhibitors as suppressors of PKD models. *Proc. Natl Acad. Sci. USA* **106**, 21819–21824 (2009).
135. Chamling, X. *et al.* Ectopic expression of human BBS4 can rescue Bardet-Biedl syndrome phenotypes in Bbs4 null mice. *PLoS ONE* **8**, e59101 (2013).
136. McCampbell, K. K. & Wingert, R. A. New tides: using zebrafish to study renal regeneration. *Transl. Res.* **163**, 109–122 (2014).
137. Genin, E., Feingold, J. & Clerget-Darpoux, F. Identifying modifier genes of monogenic disease: strategies and difficulties. *Hum. Genet.* **124**, 357–368 (2008).
138. Iatropoulos, P. *et al.* Discordant phenotype in monozygotic twins with renal coloboma syndrome and a PAX2 mutation. *Pediatr. Nephrol.* **27**, 1989–1993 (2012).
139. Zhang, Y. *et al.* BBS mutations modify phenotypic expression of CEP290-related ciliopathies. *Hum. Mol. Genet.* **23**, 40–51 (2014).
140. European Consortium for High-throughput Research in Rare Kidney Diseases. *EURenOmics* [online], http://www.eurenomics.eu (2014).
141. Bartlett, Y. K. & Coulson, N. S. An investigation into the empowerment effects of using online support groups and how this affects health professional/patient communication. *Patient Educ. Couns.* **83**, 113–119 (2011).
142. Saunier, S. *et al.* A novel gene that encodes a protein with a putative src homology 3 domain is a candidate gene for familial juvenile nephronophthisis. *Hum. Mol. Genet.* **6**, 2317–2323 (1997).

143. Hildebrandt, F. *et al.* A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. *Nat. Genet.* **17**, 149–153 (1997).
144. Otto, E. A. *et al.* Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat. Genet.* **34**, 413–420 (2003).
145. Olbrich, H. *et al.* Mutations in a novel gene, NPHP3, cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. *Nat. Genet.* **34**, 455–459 (2003).
146. Mollet, G. *et al.* The gene mutated in juvenile nephronophthisis type 4 encodes a novel protein that interacts with nephrocystin. *Nat. Genet.* **32**, 300–305 (2002).
147. Otto, E. *et al.* A gene mutated in nephronophthisis and retinitis pigmentosa encodes a novel protein, nephroretinin, conserved in evolution. *Am. J. Hum. Genet.* **71**, 1161–1167 (2002).
148. Otto, E. A. *et al.* Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. *Nat. Genet.* **37**, 282–288 (2005).
149. Delous, M. *et al.* The ciliary gene *RPGRIP1L* is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat. Genet.* **39**, 875–881 (2007).
150. Otto, E. A. *et al. NEK8* mutations affect ciliary and centrosomal localization and may cause nephronophthisis. *J. Am. Soc. Nephrol.* **19**, 587–592 (2008).
151. Davis, E. E. *et al.* TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat. Genet.* **43**, 189–196 (2011).
152. Halbritter, J. *et al.* Defects in the IFT-B component IFT172 cause Jeune and Mainzer-Saldino syndromes in humans. *Am. J. Hum. Genet.* **93**, 915–925 (2013).

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Author contributions
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