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REVIEW

# Long-Read Sequencing in CKD Diagnostics: Breaking Genomic Barriers and Expanding Global Inclusion

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Chronic kidney disease (CKD) is a major global health burden, and genetic testing is increasingly used to clarify diagnosis and guide management, particularly in inherited and early-onset disease. Short-read sequencing (SRS), including targeted gene panels, exome sequencing, and genome sequencing, provides diagnostic-grade detection of many exonic single nucleotide variants and small insertions or deletions, but important diagnostic gaps remain in genomic regions that are difficult to resolve with short reads. Long-read sequencing (LRS) addresses several of these limitations by enabling improved detection of structural variants, repeat expansions, and complex or highly homologous loci, and by providing long-range haplotype context.

This review focuses on the current and emerging clinical applications of LRS in CKD diagnostics, highlighting scenarios where LRS is most likely to add value beyond SRS, including unresolved suspected monogenic kidney disease and technically challenging genes such as *PKD1* and *MUC1*. We also discuss how improved representation of structural variation and ancestry-specific reference data may reduce uncertainty in variant interpretation for patients who are poorly represented in existing databases.

Although routine clinical implementation of LRS is still evolving, ongoing advances in laboratory workflows, bioinformatics pipelines, and reference resources are accelerating translation into nephrology practice.

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CKD remains a major global health challenge, imposing substantial economic burdens.[1-3] However, recent advancements in genomic technologies have improved our broad understanding of the genetic causes of CKD, offering opportunities for early diagnosis and targeted interventions.[4,5]

Genetics in CKD exists on a continuum, ranging from monogenic kidney diseases caused by high-penetrance variants to common CKD, where genetic variants act as risk modifiers alongside environmental factors,[6-8] including socio-economic status and lifestyle choices.[9-11] In clinical practice, monogenic kidney diseases are often suspected in conditions such as autosomal dominant polycystic kidney disease (ADPKD), most commonly caused by variants in *PKD1* or *PKD2*, although additional genes including *GANAB, DNAJB11, ALG8,* and *ALG9* are now recognized and routinely included in clinical gene panels,[12,13] Alport syndrome associated with pathogenic variants in *COL4A3–COL4A5*,[14] or nephronophthisis caused by variants in *NPHP* genes.[7] In contrast, common forms of CKD, such as diabetic kidney disease[15] or hypertensive nephropathy,[16] typically reflect polygenic risk combined with environmental exposures rather than a single causal variant. Genetic testing is therefore most frequently pursued in patients with suspected

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inherited or early-onset disease, where a single pathogenic variant is more likely to explain the phenotype.[17,18] In this diagnostic context, particularly when SRS fails to resolve complex or structurally challenging loci, LRS may offer additional diagnostic value.[19-21]

LRS, also known as third-generation sequencing, offers transformative potential in CKD genetics by enabling the precise detection of structural variants, deep intronic variants , and other complex genomic alterations that are often missed by SRS methods (Table 1).[22-24] However, the successful implementation of LRS necessitates tailored methodological workflows for DNA extraction, library preparation, and sequencing, as well as suitable computational sources, robust bioinformatics pipelines, and reference databases for variant interpretation.[25-28] Ongoing developments in the field and multidisciplinary efforts are progressively addressing these challenges, enhancing the clinical applicability of LRS.[29,30] The higher up-front cost of LRS reflects sequencing consumable costs, lower multiplexing capacity, and greater analytical complexity compared with short-read methods.[31] As the technology becomes more accessible and cost-effective in relation to its diagnostic yield and clinical impact, its adoption is expected to expand, offering promising avenues for early diagnosis and personalized treatment strategies in CKD.[32]

CKD arises from multiple subtypes influenced by over 600 identified genes, rendering it a multifaceted disorder shaped by the intricate interplay between genetic predispositions and environmental stressors, including epigenetic modifications like DNA methylation that modulate gene expression.[33-36] In patients with suspected inherited or monogenic forms of CKD, early genomic diagnosis can prevent costly and high-risk invasive procedures such as hemodialysis and kidney transplant, whereas also enabling family screening and early delivery of targeted treatments.[37] The 2022 Kidney Disease: Improving Global Outcomes Controversies Conference highlighted the substantial influence of genetics in the assessment of CKD cases, stressing the need for thorough family history evaluations, consideration of the age of CKD onset, extra-renal symptoms, and the inclusion of genetic testing in the diagnostic process.[36]

This review examines the current and emerging role of LRS in the diagnostic evaluation of CKD, with emphasis on scenarios in which LRS provides added value beyond conventional SRS. We focus on its application in suspected monogenic kidney disease, particularly in resolving structurally complex loci and repeat-associated variants, and in cases remaining unsolved after standard testing. By synthesizing recent primary studies, we aim to clarify the clinical positioning of LRS as a complementary strategy within nephrogenetic practice.

## Current Genetic Testing in CKD and Limitations

Although conventional diagnostic approaches such a biochemical test, imaging, and biopsies remain central to the evaluation of nephropathy, they often fail to establish an underlying diagnosis in a subset of patients.[38,39] An important proportion of patients frequently experience uncertainty during the

**Table 1.** Technical characteristics of short-read sequencing (SRS) and long-read sequencing (LRS) relevant to clinical genomics

| Performance metrics | SRS | LRS | References |
|---|---|---|---|
| SNV/indel performance (clinical) | F1 ≈ 0.98–0.99 (Illumina) | F1 ≈ 0.95–0.98 (PacBio HiFi); 0.90–0.96 (ONT, model-dependent) | Performance metrics derived from Genome in a Bottle benchmarking datasets[49,134,135] |
| Per-read base accuracy | High ~99.8–99.9% | Lower ~ 99.8% (PacBio HiFi); ~95–98% (ONT, chemistry/model-dependent) | Wenger *et al.*[49] |
| Cost-effectiveness (clinical context dependent) | Lower cost; cost-efficient for routine SNV detection | Higher upfront cost; may reduce downstream testing in structurally complex or unresolved cases | Groopman *et al.*[44] |
| Throughput | High (hundreds–thousands of samples/run) | Medium (lower sample multiplexing; improving) | Logsdon *et al.*[22] and Goodwin *et al.*[136] |
| Time efficiency | **Moderate** (batch-based workflows, delayed results) | High (real-time sequencing and analysis possible) | Logsdon *et al.*[22], Quick *et al.*[137], and De Coster *et al.*[138] |
| SVs detection | Some (mostly CNVs; breakpoint resolution poor) | Yes (balanced SVs, repeat-associated SVs, complex rearrangements) simultaneous with sequencing. | Chaisson *et al.*[50] and Sedlazeck *et al.*[139] |
| Epigenetic modifications detection | Indirect (separate assays required) | Direct (simultaneous with sequencing) | Simpson *et al.*[140] and Liu *et al.*[141] |
| Maximum read length | ~150–300 bp | 10–25 kb (PacBio HiFi); > 100 kb (ONT) | Logsdon *et al.*[22], Amarasinghe *et al.*[27], Wenger *et al.*[49], and Jain *et al.*[142] |

bp, base pairs; CNV, copy number variant; F1, F1 score (harmonic mean of precision and recall); indel, insertion/deletion; ONT, Oxford Nanopore Technologies; SNV, single nucleotide variant; SV, structural variant.
Performance metrics are derived from published benchmarking studies and reflect variant-type–specific capabilities. SRS remains highly accurate for SNVs and small insertions/deletions (indels) in well-characterized genomic regions, whereas LRS provides advantages for SV detection, repeat expansions, complex or highly homologous loci, and haplotype resolution. Epigenetic information can be obtained using SRS through dedicated assays requiring separate workflows, whereas certain LRS platforms enable direct detection of base modifications from native DNA within a single sequencing run.[49,81,136,143,144]

diagnostic journey and express a desire for earlier and clearer information, particularly in the absence of a known family history.[40] Patient and caregiver studies in inherited kidney disease, including ADPKD, highlight the perceived value of early genetic information and the emotional burden associated with diagnostic uncertainty and unclear prognosis.[41-43] These observations highlight the clinical consequences of delayed or uncertain etiological diagnosis and underscore the potential value of earlier genetic evaluation in selected patients with CKD, particularly when conventional clinical assessment fails to establish a clear cause.

Current genetic testing options for CKD include targeted gene panels, exome sequencing, and genome sequencing which are routinely performed using next-generation SRS platforms and enable parallel interrogation of multiple kidney disease genes.[44,45] Sanger sequencing remains a cost-effective and accurate method for confirmatory testing or targeted analysis of known familial variants but is not used for broad, first-line genetic evaluation.[46] Copy number variants are now typically detected through SRS-based approaches rather than chromosomal microarray analysis.[47] These approaches provide diagnostic-grade detection of single nucleotide variants and small insertions or deletions, and have become the standard of care for many suspected inherited kidney diseases. However, the short-read lengths (typically ~150 base pairs) limits accurate variant detection in certain genomic contexts. Structural variants (SVs), repeat expansions, guanine-cytosine (GC)-rich regions, and highly homologous or duplicated loci remain challenging to resolve, contributing to unresolved variants in a subset of Mendelian disorders. These limitations provide the clinical rationale for exploring complementary sequencing approaches, such as LRS, in selected patients with CKD, where standard testing has failed to establish a molecular diagnosis.[48-50]

A relevant example illustrating this analytical difficulty is the clinically important gene *PKD1*. *PKD1* represents a technically challenging locus because of the presence of 6 highly homologous pseudogenes spanning exons 1 to 33, which share approximately 97% sequence identity with the functional gene. Although clinical testing of *PKD1* using SRS is widely implemented and supported by specialized laboratory workflows, these approaches remain technically complex and may fail to resolve certain intronic, structural, or splice-altering variants. Recent studies incorporating targeted LRS have demonstrated improved resolution of *PKD1* variants, including detection of atypical splice variants and more accurate differentiation from pseudogene sequences, supporting an integrative role for LRS in selected unresolved or complex cases.[23,51] Our understanding of CKD mechanisms and causes has advanced in tandem with emerging technologies, from the first genome-wide association studies in 2007 to the adoption of LRS in 2020, highlighting significant progress in unravelling this complex disease and offering hope for more precise diagnostics and deeper insight in the years ahead (Figure 1).[48-50]

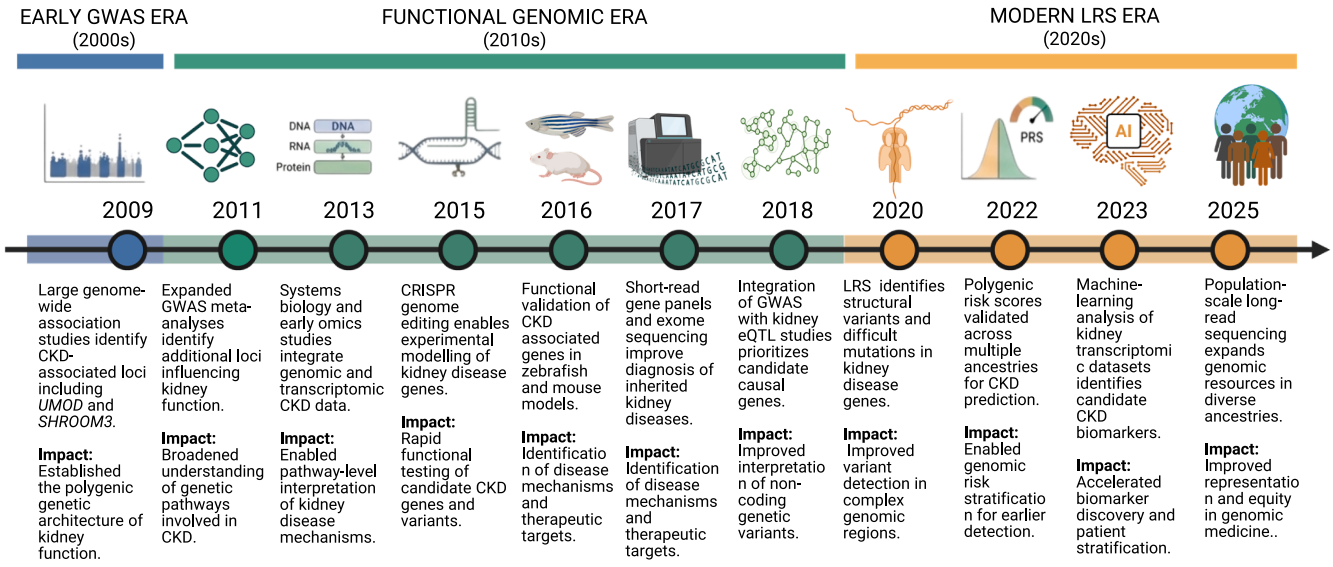


**Figure 1.** Timeline of major genomic advancements in chronic kidney disease (CKD) research and clinical diagnostics. The timeline illustrates the progression from early genome-wide association studies (GWAS) to the adoption of short-read sequencing (SRS)-based target gene panels and exome sequencing of Mendelian CKD, and more recently to long-read (LRS) technologies. Key milestones highlight the evolving contributions of genomics to understanding CKD mechanisms, improving diagnostic yield, and enabling more precise molecular characterization. CRISPR, clustered regularly interspaced short palindromic repeats; ES, exome sequencing; GS, genome sequencing; LRS, long-read sequencing; PRS, polygenic risk score; SRS, short-read sequencing[52,53-62] Created with BioRender (BioRender.com).

Reported diagnostic yields for short-read genomic sequencing in established CKD cohorts range from approximately 10% to 30% in adult populations. In this context, diagnostic yield refers to the proportion of individuals in whom sequencing identifies a pathogenic or likely pathogenic variant that explains the patient's clinical phenotype. Primary studies applying exome sequencing in adult CKD cohorts have reported diagnostic rates between approximately 9% and 24%, with higher yields observed in selected groups such as early-onset disease, positive family history, or syndromic presentations.[44,63,64] In some cases, genomic diagnosis leads to reclassification of the clinical diagnosis and influences management decisions, including avoidance of inappropriate immunosuppression or unnecessary invasive investigations. Although whole genome sequencing (WGS) can improve variant detection compared with exome sequencing in technically challenging regions, many diagnostic gaps persist, reflecting the intrinsic limitations of short-read technologies rather than gene content alone.[65,66]

LRS overcomes many of these constraints by generating extended reads that span complex genomic regions, enabling improved alignment, variant phasing, and structural variant detection. The key technical differences between SRS and LRS that underpin these diagnostic advantages are illustrated in Figure 2. LRS has shown several applications in the clinical discovery of recently identified pathogenic gene variants in human diseases, investigating genetic disorders with previously unknown genetic cause or strongly suspected disease loci.[67,68]

## Clinical Applications of LRS in Kidney Disease

There are currently 2 major LRS technologies: single molecule real-time and nanopore-based sequencing platforms. Single molecule real-time sequencing, developed by Pacific Biosciences (PacBio, Menlo Park, CA) was the first widely adopted LRS technology. It facilitates the direct observation of DNA polymerase activity as it synthesizes new DNA. Fluorescently labelled bases incorporated by the polymerase, allow

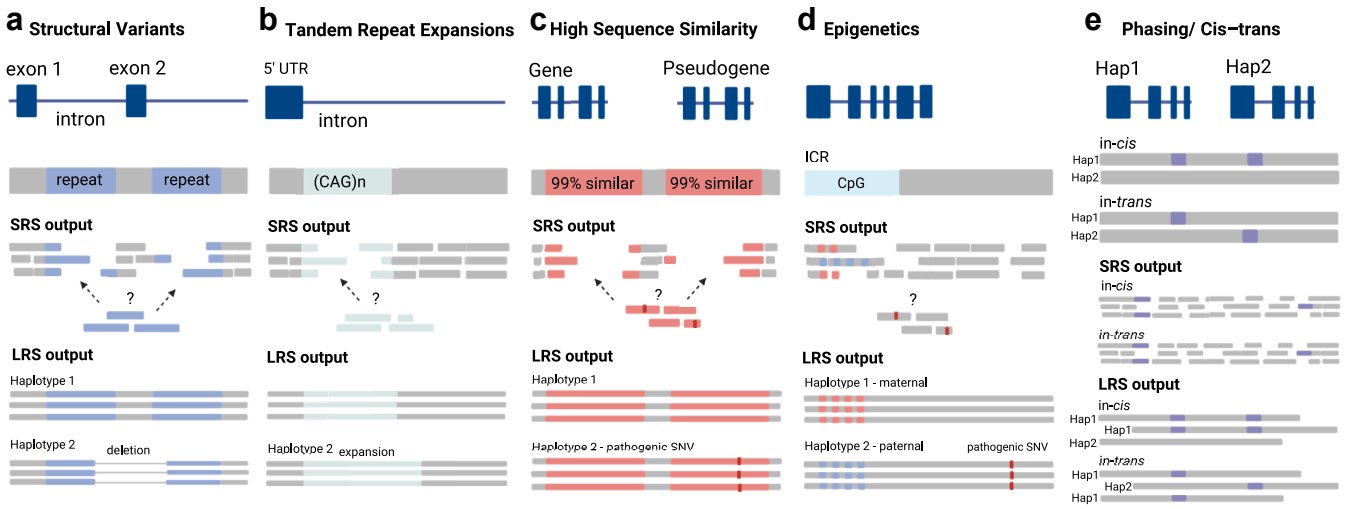


**Figure 2.** Advantages of long-read sequencing (LRS) compared with short-read sequencing (SRS) in complex genetic regions. Short reads often fail to capture the extended genetic context, which complicates accurate alignment. In contrast, LRS generates extended reads, enabling precise placement and improved resolution of such regions. (a) LRS demonstrates enhanced capacity to identify structural variants (SVs) relative to SRS, particularly in difficult genomic areas like repetitive DNA sequences (depicted in blue), which frequently contribute to SV formation. (b) LRS facilitates superior sequencing and alignment of short tandem repeat (STR) regions (shown in green) compared with SRS, allowing precise identification of STR expansions. Notable genes with STR expansions linked to disorders include *PKD1* (autosomal dominant polycystic kidney disease),[69] *NPHP1* (nephronophthisis),[70] and *COL4A5* (Alport syndrome).[71] (c) LRS improves the mapping and coverage of genomic regions with high sequence homology (illustrated in red), which pose challenges for SRS. This capability supports the distinction between genes and their pseudogenes, enhancing the detection of variations in these complex regions. Examples of disease-related genes in such homologous regions include *PKD2* (polycystic kidney disease),[72] *CFHR5* (complement factor H-related 5 glomerulopathy),[73] and *MYH9*.[74] (d) The ability to sequence native DNA strands using LRS allows simultaneous evaluation of base modifications, such as distinguishing methylated cytosines (marked in red) from unmethylated cytosines (marked in blue) within cytosine-guanine dinucleotides (CpGs). This can be paired with phasing data to explore imprinted regions exhibiting parent-specific DNA methylation. Examples of imprinted genes with methylation patterns linked to CKD include *WT1* (altered methylation in kidney development defects)[75] and *HNF1B* (methylation changes in renal cysts and diabetes syndrome).[76] (e) LRS enables direct haplotype phasing, allowing determination of whether multiple variants occur on the same chromosome (in *cis*) or on opposite chromosomes (in *trans*). This information is critical for interpreting variant interactions that modify disease risk. For example, at the *APOL1* locus, the p.N264K (M1) variant has been shown to confer a strong protective effect against G2-associated kidney disease when present on the same haplotype, highlighting the importance of accurate phasing when evaluating *APOL1* risk configurations in CKD genetics.[77,78] CKD, chronic kidney disease. Created with BioRender (BioRender.com).

for real-time detection through zero-mode waveguides. PacBio LRS platforms currently include the Sequel IIe, Revio, and Vega sequencers.[22]

Nanopore-based sequencing, developed by Oxford Nanopore Technologies (ONT; Oxford, UK), has grown significantly since its first commercial release in 2014. This method uses a membrane with embedded nanopores to measure ionic current changes as DNA or RNA passes through. During library preparation, motor protein-tagged adapters are attached to guide the polynucleotides into the pores. As the template strand translocates through the nanopore, unique disruptions in current flow correspond to each nucleotide, enabling real-time sequencing. ONT platforms include MinION, GridION, and PromethION, which are applicable in both basic and clinical research.[79,80]

LRS can significantly enhance the detection and characterization of SVs, improve haplotype resolution, accurately differentiate pseudogenes, and sequence tandem repeat expansions (Figure 2). Thus, LRS holds the potential to increase the diagnostic yield of genetic testing and facilitate the discovery of novel disease-associated genes.[67]

A pilot study conducted across All of Us research program sequencing centers applied LRS to a set of control samples to evaluate platform performance and reproducibility. This study demonstrated that LRS substantially improved genome assembly quality and structural variant detection compared with SRS, with PacBio HiFi reads achieving the highest single-nucleotide variant accuracy and ONT sequencing excelling in structural variant identification across medically relevant genomic regions.[81]

To date, published applications of LRS in kidney disease can be broadly categorized into the following 3 domains: (i) resolution of technically complex loci, (ii) detection of repeat-associated pathogenic variants (including variable number of tandem repeats [VNTRs] and mobile element insertions), and (iii) improved structural variant detection and haplotype phasing in unresolved or ambiguous cases.[22]

### ADPKD (PKD1/PKD2)

A recent study investigated families with typical ADPKD, who lacked a genetic diagnosis after standard testing. By using a combination of short-read and long-read genome sequencing, the authors identified an atypical splice variant impacting *PKD1* in most previously unresolved families, with multiple pathogenic changes located in noncoding regions that are not captured by standard workflows and/or are difficult to interpret without transcript or splicing assessment.[4] More recently, targeted long-read approaches have been evaluated as clinically implementable assays for ADPKD, demonstrating improved resolution of *PKD1*/*PKD2* variant detection (including difficult *PKD1* calls) and supporting the role for LRS in complex or unresolved cases.[23,72] These studies align with the well-known technical constraint that *PKD1* pseudogene homology can complicate alignment and variant attribution using short reads, motivating long-read, or long-range polymerase chain reaction (PCR)–based solutions in selected cases[51] (Figure 2c).

### Autosomal Dominant Tubulointerstitial Kidney Disease (MUC1)

In a Japanese cohort of suspected patients with autosomal dominant tubulointerstitial kidney disease (ADTKD), LRS identified frameshift variants in the GC-rich *MUC1* known as VNTR that were missed or incompletely resolved by short-read approaches (Figure 2b), supporting the role of LRS for this repeat-associated diagnostic blind spot.[82] The VNTR region, a GC-rich (> 80%), repetitive 60-nucleotide sequence, leads to frameshift variants (e.g., 27dupC) that produce a toxic MUC1fs protein, evading standard SRS because of alignment issues.[83] Consistent with this, targeted single molecule real-time LRS has been shown to enable complete VNTR assembly and precise positioning of causative *MUC1* variants, providing an alternative to specialized non-SRS assays in ADTKD-MUC1.[84]

### APOL1-Associated Nephropathy (Phasing/cis–trans Interpretation)

The long contiguous reads generated by LRS enable direct haplotype phasing of multiple variants within the same gene, allowing determination of whether pathogenic variants are in *cis* or *trans* (Figure 2e). This information can materially change interpretation when variant combinations modify disease risk.[22,49] At the *APOL1* locus, a large human genetics study reported a strong protective effect of the p.N264K (M1) variant against G2-associated kidney disease, emphasizing the importance of accurately resolving haplotypes when interpreting *APOL1* risk configurations.[85] Because long reads can phase across the locus directly on single DNA molecules, LRS provides a practical route to establish whether protective and risk alleles occur on the same haplotype in an individual, something that can be challenging with short reads depending on distance/coverage.[77,78]

### Structural Variants and Complex Rearrangements in Inherited Kidney Disease

Structural variants (SVs) are a major, underdetected contributor to genetic kidney disease, particularly when breakpoints fall in repeat-rich regions, segmental duplications, or when rearrangements are complex (Figure 2a). In a nephronophthisis-related ciliopathy

(Senior–Løken syndrome),[24] long-read technologies were used to resolve an otherwise hidden mobile element insertion with downstream splice consequences, illustrating the value of long reads for "cryptic" structural variation missed by routine testing.[86] For kidney tubulopathies, LRS has also been used to uncover previously undetected intronic/splice-altering variants in suspected Gitelman syndrome, demonstrating how LRS can recover missing pathogenic variation after standard approaches.[87]

### Epigenetic Profiling and Regulatory Variation in CKD

LRS generates millions of reads in real-time and can be performed without PCR amplification, thereby reducing amplification-related biases such as GC-content bias, preferential amplification of shorter fragments, allelic imbalance, and dropout of repetitive or structurally complex genomic regions.[32] Because DNA is sequenced in its native state, LRS also enables the direct detection of base modifications including cytosine-guanine dinucleotides methylation[88,89] (Figure 2d). The use of LRS for epigenetic profiling facilitates the understanding of the regulatory mechanisms governing cellular gene expression and integrates the impacts of genetic variation and environmental influences. Heritability studies have shown that methylation explains a larger share of kidney disease heritability than gene expression.[35,90,91] As the application of LRS technology expands, it is expected to further our understanding of molecular mechanisms underlying kidney dysfunction.

### Detectability Versus Diagnostic Yield

Despite these clear technical advantages, it is important to distinguish improved variant detectability from overall diagnostic yield. Although LRS offers clear technical advantages in variant detectability and genomic resolution, improved technical detection does not necessarily translate into substantially higher diagnostic yields across all CKD cohorts. In unselected adult CKD populations, diagnostic yields achieved with targeted gene panels, exome sequencing, and short-read WGS are often comparable, reflecting the fact that many genetic kidney diseases are caused by exonic variants in a relatively limited number of well-characterized genes that are already detectable using short-read technologies.

Consequently, the additional variants uniquely detectable by LRS, such as deep intronic variants, complex structural rearrangements, or repeat-associated variants are expected to account for a smaller proportion of diagnoses and may be rare at the population level. The clinical value of LRS therefore lies less in increasing overall diagnostic yield in broad CKD cohorts, and more in its ability to resolve diagnostically challenging cases, clarify ambiguous findings, and provide definitive molecular diagnoses in patients who remain undiagnosed after standard testing. Current evidence supports LRS primarily as a complementary or reflex strategy, particularly when a monogenic etiology remains strongly suspected in SRS-negative diagnostic pathways.[19]

To date, published data on LRS in kidney disease derive primarily from selected patient groups, those with unresolved suspected monogenic disease or technically challenging loci, rather than unselected CKD populations. No published study has applied genome-wide LRS to a consecutively enrolled or unselected CKD cohort, and truly blinded head-to-head comparisons of SRS and LRS diagnostic yield in kidney disease remain lacking. Del Gobbo and Boycott[19] performed one of the most systematic comparisons of LRS versus SRS in rare disease broadly, demonstrating incremental diagnostic yield in SRS-negative cases; however, their cohort was not kidney-specific.

### LRS Limitations

A recognized limitation of LRS is its comparatively lower per-base coverage in whole-genome applications relative to targeted gene panels or exome sequencing, which may limit sensitivity for detecting low-level mosaic variants. Detection of mosaic variants is inherently challenging because they often occur at low variant allele fractions, and most existing mosaic variant detection algorithms were originally developed for SRS, with dedicated tools for long-read data only recently emerging.[92] Mosaic pathogenic variants have been identified in a proportion of patients with ADPKD who had negative standard genetic testing, highlighting the clinical relevance of mosaicism in CKD context.[93]

Detection of variants generally requires high read depth, which, currently is more readily achieved with short-read targeted approaches. Accordingly, LRS should be viewed as complementary rather than universally optimal, with its use guided by the specific clinical question and previous testing results.

## Strategies for LRS in CKD Diagnostics

In clinical nephrogenetics, the choice between WGS-LRS and targeted long-read approaches is guided by the underlying clinical question.[94] WGS, whether performed using short-read or long-read platforms, provides comprehensive genome-wide analysis and enables hypothesis-free variant discovery.[95–97] However, because sequencing resources are distributed across the entire genome, WGS typically achieves lower per-locus depth compared with targeted enrichment approaches, which concentrate sequencing coverage on selected

regions.[95] Consequently, targeted strategies may offer higher sensitivity for specific loci at lower cost, whereas WGS provides broader discovery potential at the expense of increased data volume, analytical complexity, and overall resource requirements.[98]

Owing to higher cost and historically higher per-read error rates associated with LRS relative to SRS, target enrichment techniques have gained prominence for enhancing the capture of disease-relevant genomic regions while addressing the issue of error rates, for example, those associated with ONT platforms.[99] In a study, targeted LRS was applied to 10 individuals with suspected recessive or X-linked Mendelian diseases whose molecular diagnosis was incomplete after previous testing. Targeted LRS improved diagnostic yield by identifying structural, intronic, and complex variants that had been missed by earlier methods.[100] Common enrichment strategies used alongside LRS include the following: adaptive sampling (AS), hybridization-based capture, amplicon-based enrichment via long-range PCR (LR-PCR), and clustered regularly interspaced short palindromic repeats (CRISPR)-guided techniques, each with distinct advantages, limitations, and applications in CKD research.

### Real-Time Enrichment – AS

Specifically developed for ONT sequencers, a real-time, molecule-by-molecule selective sequencing approach, also known as the "Read Until" technique, was introduced as an alternative to conventional enrichment approaches.[101] During sequencing, DNA strands translocate through nanopores, generating real-time electrical current signals that are decoded into nucleotide sequences through basecalling and subsequently aligned to a reference genome, with files specifying the genomic coordinates of target regions. The system provides real-time feedback to the sequencing pore, allowing fragments of interest to continue sequencing, whereas noninformative DNA is selectively ejected, freeing the pore for other molecules.[101] Advantages of AS include cost efficiency, as it enables background depletion and target enrichment in a single run without additional hardware, and flexibility for real-time adjustments, which is ideal for low-input samples without the need for amplification.[102]

In CKD research, AS has shown promise for real-time enrichment of nephrogenetic regions, as demonstrated in a 2025 study that implemented a customized AS approach, referred to as Cornetto, to specifically enrich and assemble the *MUC1* locus in VNTR region.[103] However, AS is not error-free; a 2022 study highlighted that AS could suffer from inaccurate strand ejection because of mapping errors, leading to false negative rejections (where target molecules are ejected, though observed at low occurrence), off-target sequencing, and reduced enrichment efficiency, particularly for short DNA molecules or large reference databases. Additionally, its reliance on high-performance computing, often requiring costly graphics processing units, can limit throughput in resource-constrained settings.[104]

### Hybridization-Based Capture

Hybridization-based capture commonly is used with both ONT and PacBio platforms. This method excels in covering larger genomic regions (e.g., up to megabases) with high specificity and uniformity, making it suitable for comprehensive variant detection in complex loci which makes the method suitable for clinical application.[30] However, this method also brings important disadvantages, such as higher costs for probe design and more time-consuming, error-prone library preparation. An extended and often complicated library construction protocol is needed, which involves multiple PCR amplification steps that limits the length of DNA captured and can introduce biases in repetitive or GC-rich areas. The coverage drops significantly in extreme GC-content regions, performing worse than SRS, next-generation WGS in regions of extreme GC content.[95]

A 2021 study applied hybridization capture with PacBio LRS to mismatch repair genes, achieving 98% on-target reads and superior coverage in repetitive loci.[67] In patients with ADPKD, hybridization-based capture combined with PacBio LRS has been used to resolve *PKD1* variants, achieving higher diagnostic yields by accurately distinguishing true genetic variants from pseudogene artifacts.[23]

In CKD diagnostics, hybridization-based capture is most useful for known disease loci where increased coverage can resolve complex or duplicated regions.

### Enzyme-Guided Enrichment – CRISPR-Cas Approaches

Because of its high programmability and specificity, the CRISPR-Cas9 system has been used in a wide variety of biotechnological applications that involve validate CKD-associated variant,[52] genome editing, gene therapy, and targeted sequencing.[105,106] CRISPR-guided enrichment uses Cas9 or similar enzymes to cleave and isolate target regions, enabling precise enrichment of native DNA without amplification.[107] Although in CKD context CRISPR-based enrichment has not been attempted, studies in other fields have shown that it has strong potential.

The CRISPR-guided enrichment process necessitates meticulous optimization of guide RNAs and entails risks of incomplete cleavage in complex samples, requiring substantial technical expertise and financial investment for synthesis. These factors currently

constrain the routine application of this approach in clinical settings, including its potential use in CKD diagnostics.[108]

## Amplicon-Based Enrichment – LR-PCR

LR-PCR amplifies large DNA targets using specialized polymerases, followed by LRS to generate error-corrected consensus reads. Its advantages include procedural simplicity and compatibility with PacBio circular consensus sequencing, whereas limitations involve PCR bias, artifacts in homologous regions, and failure to detect certain SVs.[51] In CKD diagnostics, LR-PCR has been used to detect *MUC1* frameshift variants in ADTKD; notably, a 2022 study enhanced diagnostic accuracy by combining LR-PCR with ONT sequencing in clinically diagnosed patients with ADTKD.[82]

Selecting the optimal enrichment strategy requires consideration of target complexity, sample quality, and sequencing platform. For CKD, hybridization and LR-PCR are widely adopted and used for their robustness in duplicated regions, whereas CRISPR-based enrichment could further improve cost-effectiveness and precision for larger panels. Together, these enrichment strategies highlight that targeted LRS approaches are complementary tools in CKD diagnostics, best applied in selected clinical contexts rather than as universal replacements for WGS.[19,23]

## Reference Bias, SV Representation, and Underrepresented Population

Genomic research plays an indirect but critical role in improving clinical care by expanding variant discovery and reference databases, particularly through the inclusion of diverse and historically underrepresented populations.[109] Publicly available genetic variation datasets, like the widely used Genome Aggregation Database, lack population-specific data for many indigenous and First Nations groups.[110] Large-scale research sequencing efforts enable the identification of population-specific single nucleotide variants and structural variants, refine allele frequency estimates, and reduce uncertainly in variant interpretation, especially for patients whose ancestry is poorly represented in existing databases.[109,111,112] However, the translation of research genomics into routine clinical management remains an evolving process and is not yet fully optimized, particularly for complex disorders such as CKD. The practical expansion of genomic access in remote and underserved communities is more likely to be achieved in the near term through centralized SRS workflows, including sample batching and transport to core sequencing facilities, than through point-of-care LRS. Portable ONT devices such as the MinION are well suited to targeted or surveillance applications, but their use for large-scale population genomics in remote areas remains logistically constrained. The more specific contribution of LRS in indigenous and underrepresented populations is in structural variant discovery and epigenetic characterization, capabilities that are not accessible with short-read methods. These advancements represent incremental but meaningful progress in CKD research and clinical care, with LRS contributing complementary capabilities that are not achievable with SRS methods alone.

It should be noted that the advantage of LRS in SV detection is not specific to any population. However, in populations with limited SRS-based reference data, such as many indigenous and First Nations groups, LRS *de novo* assembly provides a reference-independent approach to identify structural variation that would be missed by SRS alignment pipelines reliant on existing reference genomes. This has been demonstrated empirically — Reis *et al.*,[113] using Nanopore LRS, identified over 160,000 SVs unique to Aboriginal Australians not present in the Genome Aggregation Database, illustrating the complementary contribution of LRS to population genomics in this context. The primary solution to database underrepresentation, however, remains broader inclusion of diverse populations in well-funded SRS programs.

By expanding structurally resolved genomic datasets across ancestries, LRS may enhance the accuracy of variant interpretation and contribute to reducing diagnostic uncertainty, thereby potentially mitigating health inequities in indigenous communities globally.

### *Clinical Integration and Implementation Considerations*

A diagnostic workflow using LRS technology relies on close collaboration across multiple fields, including healthcare, molecular biology, biotechnology, bioinformatics, and genetics. The need for interdisciplinary collaboration is crucial, and the integration of genetic testing into CKD care faces multiple systemic and practical challenges.

### *Economic Aspects and Access*

Genetic testing enables timely and accurate diagnoses in nephrology, informing treatment decisions and potentially reducing costs by minimizing prolonged diagnostic investigations and ineffective treatments. However, its upfront costs limit widespread adoption, highlighting the need to improve access to this valuable technology. Disparities persist because of limited access to care in marginalized communities, exacerbating health inequities.[10,114,115] A study reviewed CKD-related health policies in 160 countries, finding

that fewer than 40% have dedicated strategies to solve access issues. It highlights major global disparities in CKD prevention, care, and workforce capacity. Low-income countries are particularly underserved. Authors call for urgent global action to integrate CKD into national health agendas.[116] As the access to genetic screening technology remains a challenge in many parts of the world, disparities exist based on socio-economic status, geographic location, and healthcare infrastructure, which can limit access to laboratories, technology improvements, and specialist care, for certain populations.[117]

However, since 2019, when SRS averaged ~$942 and LRS with PacBio's Sequel II cost ~$1,500, genomic sequencing costs have substantially decreased; in 2025, ONT have currently reduced the LRS costs of the human genome at 30 times depth to about $850 per individual.[118] As sequencing technologies advance, further upfront cost reductions are anticipated, fostering optimism for enhanced global accessibility and equitable integration into clinical practice.

### *Ethical Aspects*

Legal and psychological challenges associated with genetic data play a significant role in hindering progress and limiting the adoption of genetic screening.[48] Public health policies increasingly address ethical issues regarding genetic testing, such as privacy, consent, and potential discrimination based on genetic information.[119] Concerns have emerged over the accessibility of genetic testing, which may lead to information overload and potential misuse of data related to nonmedical traits.[120]

### *Technical Aspects*

Accurate interpretation of structural and intronic variants remains challenging across genomic medicine. In CKD, where SVs and deep intronic variants contribute to unresolved diagnoses in a subset of monogenic conditions, integration of LRS-derived data into shared population databases (such as Genome Aggregation Database and ClinVar) would directly support clinical variant classification.[121] Furthermore, some initiatives propose integrating LRS with artificial intelligence-based pipelines and cloud-based data-sharing frameworks to create dynamic, learning databases, but these remain in early stages.[122]

There is also a growing need for tailored analytical workflows integrating advanced bioinformatics tools to interpret the vast amount of data these techniques generate and translate it into meaningful insights and conclusions. The integration of these tools into clinical practice relies heavily on bioinformatics expertise and the development of standardized protocols.[123-125] Moreover, significant gaps remain in physicians' knowledge of genetics, further complicating its clinical application.[126] To fully realize the clinical potential of genomics in CKD, it is essential to invest in multidisciplinary collaboration among molecular biologists, bioinformaticians, and geneticists, and develop user-friendly infrastructures that support both clinical workflows and family-based screening.[18]

## Conclusions and Future Research Directions

Beyond its current role in resolving diagnostically challenging inherited kidney diseases, LRS may contribute to selected advances in CKD research and precision medicine, particularly in technically challenging diagnostic scenarios, though its broader impact will depend on continued reductions in cost and improvements in analytical pipelines. By enabling more comprehensive characterization of genetic, epigenetic, and regulatory variation, and by facilitating integration with multiomics and computational approaches, LRS may offer incremental diagnostic improvements beyond SRS in selected clinical scenarios. Its contribution to the understanding of CKD pathogenesis and informing therapeutic strategies will require further validation at scale before broader clinical translation can be achieved. Understanding the functional consequences of genetic variation is a key step toward translating genomic discoveries into clinical insight. Recent large-scale functional genomics studies in kidney tissue have begun to bridge this gap by linking genetic variants to gene expression and cellular pathways relevant to CKD. A comprehensive expression Quantitative Trait Locus mapping from 659 kidney samples identified over 9000 cell-type-specific genes influencing traits like estimated glomerular filtration rate and blood pressure, prioritizing proximal tubules for kidney function, and nominating drug-targetable genes like *ACE* for CKD therapies.[127] In parallel, another key advancement in CKD research has been the integration of multiomics approaches.[128,129] By combining genomics, transcriptomics, epigenomics, and proteomics, we are gaining a more nuanced understanding of the complex biological processes that contribute to disease progression. Multiomics not only fills gaps left by traditional genome-wide association studies, but also helps explain the so-called "missing heritability" of CKD. On the computational side, advanced machine learning algorithms are facilitating more accurate variant calling, phenotype classification. Tools such as DeepVariant and Clair3,[130] specifically optimized for LRS data, enhance the detection of rare or complex genetic variants.

Within multiomics frameworks, the integration of genomics, proteomics, metabolomics, and

transcriptomics is reshaping disease classification and prognosis. Artificial intelligence-driven models show promise for CKD risk prediction and personalized treatment planning by integrating multibiomarker panels with clinical data.[129] However, challenges remain in biomarker standardization, large-scale validation, and clinical implementation.

Continued expansion of genomic research to include diverse and historically underrepresented populations, supported by both SRS and LRS technologies, has the potential to improve variant interpretation and reduce diagnostic uncertainty across all CKD patient groups.[131]

Future research should focus on refining LRS technologies for clinical use, working to reduce its cost, identifying population-specific genetic variants to discover novel CKD markers, and enhancing bioinformatics tools to translate large-scale data into actionable clinical outcomes.[132,133] Emerging multiomics and artificial intelligence, which incorporate LRS, must address challenges in clinical validation, biomarker standardization, and the development of practical strategies for seamless incorporation into routine clinical practice, underscoring the need for continued research.[129]

Addressing social and ethical considerations is crucial, requiring strategies to build community trust, ensure privacy, promote equitable access, and implement transparent benefit-sharing, alongside public awareness campaigns and genetic counselling to empower underserved communities for informed decision-making and improved CKD prevention and care.

## DISCLOSURE

AJM declares being a member of advisory boards for Natera, GSK, and PYC, with travel support from Otsuka, and an unpaid Board member of the Australia and New Zealand Society of Nephrology. The other authors declared that they have no competing interests.

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## AUTHOR CONTRIBUTIONS

RSB surveyed the literature and wrote the first draft. JY-HH provided substantial technical advice on third-generation sequencing technologies for genetic testing. AJM and US supervised the work and helped with reviewing and revising the manuscript. All authors read and approved the final manuscript.

## REFERENCES

1. Francis A, Harhay MN, Ong ACM, et al. Chronic kidney disease and the global public health agenda: an international consensus. *Nat Rev Nephrol*. 2024;20:473–485. https://doi.org/10.1038/s41581-024-00820-6
2. Randall S, Lee CMY, Thomas E, et al. Estimating the cost of chronic kidney disease in Australia. *BMC Health Serv Res*. 2024;24:1468. https://doi.org/10.1186/s12913-024-11953-6
3. Herrington WG, Judge PK, Grams ME, Wanner C. Chronic kidney disease. *Lancet*. 2026;407:90–104. https://doi.org/10.1016/S0140-6736(25)01942-7
4. Hort Y, Sullivan P, Wedd L, et al. Atypical splicing variants in PKD1 explain most undiagnosed typical familial ADPKD. *NPJ Genom Med Atypi*. 2023;8:16. https://doi.org/10.1038/s41525-023-00362-z
5. Robert T, Raymond L, Dancer M, et al. Beyond the kidney biopsy: genomic approach to undetermined kidney diseases. *Clin Kidney J*. 2024;17:sfad099. https://doi.org/10.1093/ckj/sfad099
6. Jefferis J, Hudson R, Lacaze P, et al. Monogenic and polygenic concepts in chronic kidney disease (CKD). *J Nephrol*. 2024;37:7–21. https://doi.org/10.1007/s40620-023-01804-8
7. Hildebrandt F. Genetic kidney diseases. *Lancet*. 2010;375:1287–1295. https://doi.org/10.1016/S0140-6736(10)60236-X
8. Groopman EE, Povysil G, Goldstein DB, Gharavi AG. Rare genetic causes of complex kidney and urological diseases. *Nat Rev Nephrol*. 2020;16:641–656. https://doi.org/10.1038/s41581-020-0325-2
9. Yun HR, Kim H, Park JT, et al. Obesity, metabolic abnormality, and progression of CKD. *Am J Kidney Dis*. 2018;72:400–410. https://doi.org/10.1053/j.ajkd.2018.02.362
10. Garcia-Garcia G, Jha V. World kidney day steering C. CKD in disadvantaged populations. *Kidney Int*. 2015;87:251–253. https://doi.org/10.1038/ki.2014.369
11. Messing M, Torres JA, Holznecht N, Weimbs T. Trigger warning: how modern diet, lifestyle, and environment pull the trigger on autosomal dominant polycystic kidney disease progression. *Nutrients*. 2024;16:3281. https://doi.org/10.3390/nu16193281
12. Harris PC, Torres VE. Genetic mechanisms and signaling pathways in autosomal dominant polycystic kidney disease. *J Clin Invest*. 2014;124:2315–2324. https://doi.org/10.1172/JCI72272
13. Cornec-Le Gall E, Ong ACM. Genetic testing in autosomal dominant polycystic kidney disease: why it matters in 2025. *Clin Kidney J*. 2025;18(Suppl 2):ii17–ii25. https://doi.org/10.1093/ckj/sfaf331
14. Savige J. Heterozygous pathogenic COL4A3 and COL4A4 variants (autosomal dominant Alport syndrome) are common, and not typically associated with end-stage kidney failure, hearing loss, or ocular abnormalities. *Kidney Int Rep*. 2022;7:1933–1938. https://doi.org/10.1016/j.ekir.2022.06.001

15. Sandholm N, Cole JB, Nair V, et al. Genome-wide meta-analysis and omics integration identifies novel genes associated with diabetic kidney disease. *Diabetologia*. 2022;65:1495–1509. https://doi.org/10.1007/s00125-022-05735-0

16. Delrue C, Speeckaert MM. Beyond blood pressure: emerging pathways and precision approaches in hypertension-induced kidney damage. *Int J Mol Sci*. 2025;26:7606. https://doi.org/10.3390/ijms26157606

17. Knoers N, Antignac C, Bergmann C, et al. Genetic testing in the diagnosis of chronic kidney disease: recommendations for clinical practice. *Nephrol Dial Transplant*. 2022;37:239–254. https://doi.org/10.1093/ndt/gfab218

18. Jefferis J, Mallett AJ. Exploring the impact and utility of genomic sequencing in established CKD. *Clin Kidney J*. 2024;17:sfae043. https://doi.org/10.1093/ckj/sfae043

19. Del Gobbo GF, Boycott KM. The additional diagnostic yield of long-read sequencing in undiagnosed rare diseases. *Genome Res*. 2025;35:559–571. https://doi.org/10.1101/gr.279970.124

20. Mallawaarachchi A, McCarthy H, Forbes TA, et al. Enhancing diagnostic outcomes in kidney genetic disorders: the KidGen national kidney genomics study protocol. *BMC Nephrol*. 2025;26:51. https://doi.org/10.1186/s12882-024-03926-y

21. Negi S, Stenton SL, Berger SI, et al. Advancing long-read nanopore genome assembly and accurate variant calling for rare disease detection. *Am J Hum Genet*. 2025;112:428–449. https://doi.org/10.1016/j.ajhg.2025.01.002

22. Logsdon GA, Vollger MR, Eichler EE. Long-read human genome sequencing and its applications. *Nat Rev Genet*. 2020;21:597–614. https://doi.org/10.1038/s41576-020-0236-x

23. Sun Q, Xu P, Mao A, et al. Targeted long-read sequencing enables higher diagnostic yield of ADPKD by accurate PKD1 genetic analysis. *NPJ Genom Med*. 2025;10:22. https://doi.org/10.1038/s41525-025-00477-5

24. de Bruijn SE, Ingeborgh van den Born L, Derks R, et al. Long-read technologies identify a hidden LINE-1/ERV1 insertion in IQCB1 as causative variant for Senior-Loken syndrome. *NPJ Genom Med*. 2025;10:33. https://doi.org/10.1038/s41525-025-00490-8

25. Wang Y, Zhao Y, Bollas A, Wang Y, Au KF. Nanopore sequencing technology, bioinformatics and applications. *Nat Biotechnol*. 2021;39:1348–1365. https://doi.org/10.1038/s41587-021-01108-x

26. Trigodet F, Lolans K, Fogarty E, et al. High molecular weight DNA extraction strategies for long-read sequencing of complex metagenomes. *Mol Ecol Resour*. 2022;22:1786–1802. https://doi.org/10.1111/1755-0998.13588

27. Amarasinghe SL, Su S, Dong X, Zappia L, Ritchie ME, Gouil Q. Opportunities and challenges in long-read sequencing data analysis. *Genome Biol*. 2020;21:30. https://doi.org/10.1186/s13059-020-1935-5

28. Amarasinghe SL, Ritchie ME, Gouil Q. long-read-tools.org: an interactive catalogue of analysis methods for long-read sequencing data. *GigaScience*. 2021;10:giab003. https://doi.org/10.1093/gigascience/giab003

29. LoTempio J, Delot E, Vilain E. Benchmarking long-read genome sequence alignment tools for human genomics applications. *PeerJ*. 2023;11:e16515. https://doi.org/10.7717/peerj.16515

30. Leung AW, Leung HC, Wong CL, et al. ECNano: a cost-effective workflow for target enrichment sequencing and accurate variant calling on 4800 clinically significant genes using a single MinION flowcell. *BMC Med Genomics*. 2022;15:43. https://doi.org/10.1186/s12920-022-01190-3

31. Adewale BA. Will long-read sequencing technologies replace short-read sequencing technologies in the next 10 years? *Afr J Lab Med*. 2020;9:1340. https://doi.org/10.4102/ajlm.v9i1.1340

32. Oehler JB, Wright H, Stark Z, Mallett AJ, Schmitz U. The application of long-read sequencing in clinical settings. *Hum Genomics*. 2023;17:73. https://doi.org/10.1186/s40246-023-00522-3

33. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol*. 2016;12:133–146. https://doi.org/10.1038/nrneph.2015.205

34. Liu H, Abedini A, Ha E, et al. Kidney multiome-based genetic scorecard reveals convergent coding and regulatory variants. *Science*. 2025;387:eadp4753. https://doi.org/10.1126/science.adp4753

35. Liu H, Doke T, Guo D, et al. Epigenomic and transcriptomic analyses define core cell types, genes and targetable mechanisms for kidney disease. *Nat Genet*. 2022;54:950–962. https://doi.org/10.1038/s41588-022-01097-w

36. Köttgen A, Cornec-Le Gall E, Halbritter J, et al. Genetics in chronic kidney disease: conclusions from a Kidney Disease: improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int*. 2022;101:1126–1141. https://doi.org/10.1016/j.kint.2022.03.019

37. Jayasinghe K, Stark Z, Kerr PG, et al. Clinical impact of genomic testing in patients with suspected monogenic kidney disease. *Genet Med*. 2021;23:183–191. https://doi.org/10.1038/s41436-020-00963-4

38. de Haan A, Eijgelsheim M, Vogt L, Knoers N, de Borst MH. Diagnostic yield of next-generation sequencing in patients with chronic kidney disease of unknown etiology. *Front Genet*. 2019;10:1264. https://doi.org/10.3389/fgene.2019.01264

39. Hays T, Groopman EE, Gharavi AG. Genetic testing for kidney disease of unknown etiology. *Kidney Int*. 2020;98:590–600. https://doi.org/10.1016/j.kint.2020.03.031

40. Meuleman Y, van der Bent Y, Gentenaar L, et al. Exploring patients' perceptions about chronic kidney disease and their treatment: a qualitative study. *Int J Behav Med*. 2024;31:263–275. https://doi.org/10.1007/s12529-023-10178-x

41. Tong A, Rangan GK, Ruospo M, et al. A painful inheritance-patient perspectives on living with polycystic kidney disease: thematic synthesis of qualitative research. *Nephrol Dial Transplant*. 2015;30:790–800. https://doi.org/10.1093/ndt/gfv010

42. Odland D. A patient perspective on genetic testing for ADPKD: the lack of complete genetic information, especially early in the course of the disease, is harming adult autosomal dominant polycystic kidney disease (ADPKD) patients. *Clin J Am Soc Nephrol*. 2021;16:671–673. https://doi.org/10.2215/CJN.14051119

43. Milders J, Ramspek CL, Meuleman Y, et al. Dealing with an uncertain future: a survey study on what patients with chronic kidney disease actually want to know. *Clin Kidney J*. 2024;17:sfae225. https://doi.org/10.1093/ckj/sfae225
44. Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med*. 2019;380:142–151. https://doi.org/10.1056/NEJMoa1806891
45. Claus LR, Snoek R, Knoers N, van Eerde AM. Review of genetic testing in kidney disease patients: diagnostic yield of single nucleotide variants and copy number variations evaluated across and within kidney phenotype groups. *Am J Med Genet C*. 2022;190:358–376. https://doi.org/10.1002/ajmg.c.31995
46. Arteche-Lopez A, Avila-Fernandez A, Romero R, et al. Sanger sequencing is no longer always necessary based on a single-center validation of 1109 NGS variants in 825 clinical exomes. *Sci Rep*. 2021;11:5697. https://doi.org/10.1038/s41598-021-85182-w
47. Collins RL, Brand H, Karczewski KJ, et al. A structural variation reference for medical and population genetics. *Nature*. 2020;581:444–451. https://doi.org/10.1038/s41586-020-2287-8
48. Cocchi E, Nestor JG, Gharavi AG. Clinical genetic screening in adult patients with kidney disease. *Clin J Am Soc Nephrol*. 2020;15:1497–1510. https://doi.org/10.2215/CJN.15141219
49. Wenger AM, Peluso P, Rowell WJ, et al. Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. *Nat Biotechnol*. 2019;37:1155–1162. https://doi.org/10.1038/s41587-019-0217-9
50. Chaisson MJP, Sanders AD, Zhao X, et al. Multi-platform discovery of haplotype-resolved structural variation in human genomes. *Nat Commun*. 2019;10:1784. https://doi.org/10.1038/s41467-018-08148-z
51. Borràs DM, Vossen RHAM, Liem M, et al. DetectingPKD1-variants in polycystic kidney disease patients by single-molecule long-read sequencing. *Hum Mutat*. 2017;38:870–879. https://doi.org/10.1002/humu.23223
52. Freedman BS, Brooks CR, Lam AQ, et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat Commun*. 2015;6:8715. https://doi.org/10.1038/ncomms9715
53. Kottgen A, Glazer NL, Dehghan A, et al. Multiple loci associated with indices of renal function and chronic kidney disease. *Nat Genet*. 2009;41:712–717. https://doi.org/10.1038/ng.377
54. Kottgen A, Pattaro C, Boger CA, et al. New loci associated with kidney function and chronic kidney disease. *Nat Genet*. 2010;42:376–384. https://doi.org/10.1038/ng.568
55. Zaza G, Granata S, Rascio F, et al. A specific immune transcriptomic profile discriminates chronic kidney disease patients in predialysis from hemodialyzed patients. *BMC Med Genomics*. 2013;6:17. https://doi.org/10.1186/1755-8794-6-17
56. Khalili H, Sull A, Sarin S, et al. Developmental origins for kidney disease due to Shroom3 deficiency. *J Am Soc Nephrol*. 2016;27:2965–2973. https://doi.org/10.1681/ASN.2015060621
57. Mallett AJ, McCarthy HJ, Ho G, et al. Massively parallel sequencing and targeted exomes in familial kidney disease can diagnose underlying genetic disorders. *Kidney Int*. 2017;92:1493–1506. https://doi.org/10.1016/j.kint.2017.06.013
58. Qiu C, Huang S, Park J, et al. Renal compartment-specific genetic variation analyses identify new pathways in chronic kidney disease. *Nat Med*. 2018;24:1721–1731. https://doi.org/10.1038/s41591-018-0194-4
59. Wang GQ, Rui HL, Dong HR, et al. SMRT sequencing revealed to be an effective method for ADTKD-MUC1 diagnosis through follow-up analysis of a Chinese family. *Sci Rep*. 2020;10:8616. https://doi.org/10.1038/s41598-020-65491-2
60. Khan A, Turchin MC, Patki A, et al. 2021.
61. Yan P, Ke B, Song J, Fang X. Identification of immune-related molecular clusters and diagnostic markers in chronic kidney disease based on cluster analysis. *Front Genet*. 2023;14:1111976. https://doi.org/10.3389/fgene.2023.1111976
62. Garimella KV, Li Q, Wertz J, et al. Population-scale long-read sequencing in the all of us research program. *medRxiv*. 2025. Forthcoming.
63. Lata S, Marasa M, Li Y, et al. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. *Ann Intern Med*. 2018;168:100–109. https://doi.org/10.7326/M17-1319
64. Connaughton DM, Kennedy C, Shril S, et al. Monogenic causes of chronic kidney disease in adults. *Kidney Int*. 2019;95:914–928. https://doi.org/10.1016/j.kint.2018.10.031
65. Sadeghi-Alavijeh O, Chan MM, Doctor GT, et al. Quantifying variant contributions in cystic kidney disease using national-scale whole-genome sequencing. *J Clin Invest*. 2024:134. https://doi.org/10.1172/JCI181467
66. Byrska-Bishop M, Evani US, Zhao X, et al. High-coverage whole-genome sequencing of the expanded 1000 Genomes Project cohort including 602 trios. *Cell*. 2022;185:3426–3440.e19. https://doi.org/10.1016/j.cell.2022.08.004
67. Mastrorosa FK, Miller DE, Eichler EE. Applications of long-read sequencing to Mendelian genetics. *Genome Med*. 2023;15:42. https://doi.org/10.1186/s13073-023-01194-3
68. Xu R, Zhang M, Yang X, Tian W, Li C. Decoding complexity: the role of long-read sequencing in unraveling genetic disease etiologies. *Mutat Res Rev Mutat Res*. 2025;795:108529. https://doi.org/10.1016/j.mrrev.2024.108529
69. Ali H, Al-Mulla F, Hussain N, et al. PKD1 duplicated regions limit clinical utility of whole exome sequencing for genetic diagnosis of autosomal dominant polycystic Kidney Disease. *Sci Rep*. 2019;9:4141. https://doi.org/10.1038/s41598-019-40761-w
70. Saunier S, Calado J, Benessy F, et al. Characterization of the NPHP1 locus: mutational mechanism involved in deletions in familial juvenile nephronophthisis. *Am J Hum Genet*. 2000;66:778–789. https://doi.org/10.1086/302819
71. Arrondel C, Deschenes G, Le Meur Y, et al. A large tandem duplication within the COL4A5 gene is responsible for the high prevalence of Alport syndrome in French Polynesia. *Kidney Int*. 2004;65:2030–2040. https://doi.org/10.1111/j.1523-1755.2004.00622.x
72. Xu D, Mao A, Chen L, Wu L, Ma Y, Mei C. Comprehensive analysis of PKD1 and PKD2 by long-read sequencing in autosomal dominant polycystic kidney disease. *Clin Chem*. 2024;70:841–854. https://doi.org/10.1093/clinchem/hvae030
73. Zipfel PF, Wiech T, Stea ED, Skerka C, CFHR. CFHR Gene variations provide insights in the pathogenesis of the

kidney diseases atypical hemolytic uremic syndrome and C3 glomerulopathy. *J Am Soc Nephrol*. 2020;31:241–256. https://doi.org/10.1681/ASN.2019050515

74. Freedman BI, Hicks PJ, Bostrom MA, et al. Polymorphisms in the non-muscle myosin heavy chain 9 gene (MYH9) are strongly associated with end-stage renal disease historically attributed to hypertension in African Americans. *Kidney Int*. 2009;75:736–745. https://doi.org/10.1038/ki.2008.701
75. Dressler GR, Patel SR. Epigenetics in kidney development and renal disease. *Transl Res*. 2015;165:166–176. https://doi.org/10.1016/j.trsl.2014.04.007
76. Clissold RL, Ashfield B, Burrage J, et al. Genome-wide methylomic analysis in individuals with HNF1B intragenic mutation and 17q12 microdeletion. *Clin Epigenet*. 2018;10:97. https://doi.org/10.1186/s13148-018-0530-z
77. Gbadegesin R, Martinelli E, Gupta Y, et al. APOL1 genotyping is incomplete without testing for the protective M1 modifier p. N264K variant. *Glomerular dis*. 2024;4:43–48. https://doi.org/10.1159/000537948
78. da Silva Francisco R Jr, Punj S, Vincent L, et al. Prevalence of the M1 Modifier p.N264K in APOL1 among individuals with kidney disease undergoing commercial genetic testing in the United States. *Am J Kidney Dis*. 2025;86(5):715–718. https://doi.org/10.1053/j.ajkd.2025.07.004
79. Warburton PE, Sebra RP. Long-read DNA sequencing: recent advances and remaining challenges. *Annu Rev Genomics Hum Genet*. 2023;24:109–132. https://doi.org/10.1146/annurev-genom-101722-103045
80. Deamer D, Akeson M, Branton D. Three decades of nanopore sequencing. *Nat Biotechnol*. 2016;34:518–524. https://doi.org/10.1038/nbt.3423
81. Mahmoud M, Huang Y, Garimella K, et al. Utility of long-read sequencing for All of Us. *Nat Commun*. 2024;15:837. https://doi.org/10.1038/s41467-024-44804-3
82. Okada E, Morisada N, Horinouchi T, et al. Detecting MUC1 variants in patients clinicopathologically diagnosed with having autosomal dominant tubulointerstitial kidney disease. *Kidney Int Rep*. 2022;7:857–866. https://doi.org/10.1016/j.ekir.2021.12.037
83. Fages V, Bourre F, Larrue R, et al. Description of a new simple and cost-effective molecular testing that could simplify MUC1 variant detection. *Kidney Int Rep*. 2024;9:1451–1457. https://doi.org/10.1016/j.ekir.2024.01.058
84. Wenzel A, Altmueller J, Ekici AB, et al. Single molecule real time sequencing in ADTKD-MUC1 allows complete assembly of the VNTR and exact positioning of causative mutations. *Sci Rep*. 2018;8:4170. https://doi.org/10.1038/s41598-018-22428-0
85. Gupta Y, Friedman DJ, McNulty MT, et al. Strong protective effect of the APOL1 p.N264K variant against G2-associated focal segmental glomerulosclerosis and kidney disease. *Nat Commun*. 2023;14:7836. https://doi.org/10.1038/s41467-023-43020-9
86. Kosugi S, Terao C. Comparative evaluation of SNVs, indels, and structural variations detected with short- and long-read sequencing data. *Hum Genome Var*. 2024;11:18. https://doi.org/10.1038/s41439-024-00276-x
87. Viering D, Hureaux M, Neveling K, et al. Long-read sequencing identifies novel pathogenic intronic variants in Gitelman syndrome. *J Am Soc Nephrol*. 2023;34:333–345. https://doi.org/10.1681/ASN.2022050627
88. Yuen ZW, Srivastava A, Daniel R, McNevin D, Jack C, Eyras E. Systematic benchmarking of tools for CpG methylation detection from nanopore sequencing. *Nat Commun*. 2021;12:3438. https://doi.org/10.1038/s41467-021-23778-6
89. Fu Y, Timp W, Sedlazeck FJ. Computational analysis of DNA methylation from long-read sequencing. *Nat Rev Genet*. 2025;26:620–634. https://doi.org/10.1038/s41576-025-00822-5
90. Jones AC, Patki A, Srinivasasainagendra V, et al. A methylation risk score for chronic kidney disease: a HyperGEN study. *Sci Rep*. 2024;14:17757. https://doi.org/10.1038/s41598-024-68470-z
91. Chan K, Li X. Current epigenetic insights in kidney development. *Genes (Basel)*. 2021;12:1281. https://doi.org/10.3390/genes12081281
92. Chen L, Zheng Z, He M, et al. 2025.
93. Hopp K, Cornec-Le Gall E, Senum SR, et al. Detection and characterization of mosaicism in autosomal dominant polycystic kidney disease. *Kidney Int*. 2020;97:370–382. https://doi.org/10.1016/j.kint.2019.08.038
94. Olivucci G, Iovino E, Innella G, Turchetti D, Pippucci T, Magini P. Long read sequencing on its way to the routine diagnostics of genetic diseases. *Front Genet*. 2024;15:1374860. https://doi.org/10.3389/fgene.2024.1374860
95. Barbitoff YA, Polev DE, Glotov AS, et al. Systematic dissection of biases in whole-exome and whole-genome sequencing reveals major determinants of coding sequence coverage. *Sci Rep*. 2020;10:2057. https://doi.org/10.1038/s41598-020-59026-y
96. Souche E, Beltran S, Brosens E, et al. Recommendations for whole genome sequencing in diagnostics for rare diseases. *Eur J Hum Genet*. 2022;30:1017–1021. https://doi.org/10.1038/s41431-022-01113-x
97. Bherer C, Eveleigh R, Trajanoska K, et al. A cost-effective sequencing method for genetic studies combining high-depth whole exome and low-depth whole genome. *NPJ Genom Med*. 2024;9:8. https://doi.org/10.1038/s41525-024-00390-3
98. Pei XM, Yeung MHY, Wong ANN, et al. Targeted sequencing approach and its clinical applications for the molecular diagnosis of human diseases. *Cells*. 2023;12:493. https://doi.org/10.3390/cells12030493
99. Zhang H, Li H, Jain C, et al. Real-time mapping of nanopore raw signals. *Bioinformatics*. 2021;37(Suppl 1):i477–i483. https://doi.org/10.1093/bioinformatics/btab264
100. Miller DE, Sulovari A, Wang T, et al. Targeted long-read sequencing identifies missing disease-causing variation. *Am J Hum Genet*. 2021;108:1436–1449. https://doi.org/10.1016/j.ajhg.2021.06.006
101. Loose M, Malla S, Stout M. Real-time selective sequencing using nanopore technology. *Nat Methods*. 2016;13:751–754. https://doi.org/10.1038/nmeth.3930
102. Yang L, Lin Y, Li P, et al. A comprehensive benchmarking of adaptive sampling tools for nanopore sequencing. *Genome Biol*. 2025;26:281. https://doi.org/10.1186/s13059-025-03729-w

103. Gamaarachchi H, Stevanovski I, Hammond JM, et al. *Adaptively integrated sequencing and assembly of near-complete genomes*. 2025. Forthcoming.

104. Martin S, Heavens D, Lan Y, Horsfield S, Clark MD, Leggett RM. Nanopore adaptive sampling: a tool for enrichment of low abundance species in metagenomic samples. *Genome Biol*. 2022;23:11. https://doi.org/10.1186/s13059-021-02582-x

105. Gilpatrick T, Lee I, Graham JE, et al. Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nat Biotechnol*. 2020;38:433–438. https://doi.org/10.1038/s41587-020-0407-5

106. Hryhorowicz M, Lipinski D, Zeyland J, Slomski R. CRISPR/Cas9 immune system as a tool for genome engineering. *Arch Immunol Ther Exp (Warsz)*. 2017;65:233–240. https://doi.org/10.1007/s00005-016-0427-5

107. Mizuguchi T, Toyota T, Miyatake S, et al. Complete sequencing of expanded SAMD12 repeats by long-read sequencing and Cas9-mediated enrichment. *Brain*. 2021;144:1103–1117. https://doi.org/10.1093/brain/awab021

108. Ameur A. CRISPR and long-read sequencing: a perfect match. *CRISPR J*. 2020;3:425–427. https://doi.org/10.1089/crispr.2020.29110.aam

109. Han AL, Sands CF, Matelska D, et al. Diverse ancestral representation improves genetic intolerance metrics. *Nat Commun*. 2025;16:2648. https://doi.org/10.1038/s41467-025-57885-5

110. Gudmundsson S, Singer-Berk M, Watts NA, et al. Variant interpretation using population databases: lessons from gnomAD. *Hum Mutat*. 2022;43:1012–1030. https://doi.org/10.1002/humu.24309

111. Fatumo S, Chikowore T, Choudhury A, Ayub M, Martin AR, Kuchenbaecker K. A roadmap to increase diversity in genomic studies. *Nat Med*. 2022;28:243–250. https://doi.org/10.1038/s41591-021-01672-4

112. Easteal S, Arkell RM, Balboa RF, et al. Equitable expanded carrier screening needs indigenous clinical and population genomic data. *Am J Hum Genet*. 2020;107:175–182. https://doi.org/10.1016/j.ajhg.2020.06.005

113. Reis ALM, Rapadas M, Hammond JM, et al. The landscape of genomic structural variation in Indigenous Australians. *Nature*. 2023;624:602–610. https://doi.org/10.1038/s41586-023-06842-7

114. Recabarren J, Dominello A, Scholes-Robertson N, et al. It matters who you are and where you live: commonwealth, state and territory policies for access to care for Australians with chronic kidney disease and their caregivers. *Aust N Z J Public Health*. 2024;48:100131. https://doi.org/10.1016/j.anzjph.2024.100131

115. D'Angelo CS, Hermes A, McMaster CR, et al. Barriers and considerations for diagnosing rare diseases in indigenous populations. *Front Pediatr*. 2020;8:579924. https://doi.org/10.3389/fped.2020.579924

116. Neuen BL, Bello AK, Levin A, et al. National health policies and strategies for addressing chronic kidney disease: data from the International Society of Nephrology Global Kidney Health Atlas. *PLOS Glob Public Health*. 2023;3:e0001467. https://doi.org/10.1371/journal.pgph.0001467

117. Neale EP, Middleton J, Lambert K. Barriers and enablers to detection and management of chronic kidney disease in primary healthcare: a systematic review. *BMC Nephrol*. 2020;21:83. https://doi.org/10.1186/s12882-020-01731-x

118. Gambardella G 2025.

119. Kruse J, Mueller R, Aghdassi AA, Lerch MM, Salloch S. Genetic testing for rare diseases: a systematic review of ethical aspects. *Front Genet*. 2021;12:701988. https://doi.org/10.3389/fgene.2021.701988

120. Bowman-Smart H, Gyngell C, Mand C, Amor DJ, Delatycki MB, Savulescu J. Non-invasive prenatal testing for "non-medical" traits: ensuring consistency in ethical decision-making. *Am J Bioeth*. 2023;23:3–20. https://doi.org/10.1080/15265161.2021.1996659

121. Satam H, Joshi K, Mangrolia U, et al. Next-generation sequencing technology: current trends and advancements. *Biology (Basel)*. 2023;12:997. https://doi.org/10.3390/biology12070997

122. Espinosa E, Bautista R, Larrosa R, Plata O. Advancements in long-read genome sequencing technologies and algorithms. *Genomics*. 2024;116:110842. https://doi.org/10.1016/j.ygeno.2024.110842

123. Helal AA, Saad BT, Saad MT, Mosaad GS, Aboshanab KM. Benchmarking long-read aligners and SV callers for structural variation detection in Oxford nanopore sequencing data. *Sci Rep*. 2024;14:6160. https://doi.org/10.1038/s41598-024-56604-2

124. Chen X, Wei S, Sun C, et al. Computational tools for studying genome structural variation. *Omics A J Integr Biol*. 2025;29:36–48. https://doi.org/10.1089/omi.2024.0200

125. Sedlazeck FJ, Lee H, Darby CA, Schatz MC. Piercing the dark matter: bioinformatics of long-range sequencing and mapping. *Nat Rev Genet*. 2018;19:329–346. https://doi.org/10.1038/s41576-018-0003-4

126. White S, Jacobs C, Phillips J. Mainstreaming genetics and genomics: a systematic review of the barriers and facilitators for nurses and physicians in secondary and tertiary care. *Genet Med*. 2020;22:1149–1155. https://doi.org/10.1038/s41436-020-0785-6

127. Sheng X, Guan Y, Ma Z, et al. Mapping the genetic architecture of human traits to cell types in the kidney identifies mechanisms of disease and potential treatments. *Nat Genet*. 2021;53:1322–1333. https://doi.org/10.1038/s41588-021-00909-9

128. Liu X, Shi J, Jiao Y, et al. Integrated multi-omics with machine learning to uncover the intricacies of kidney disease. *Brief Bioinform*. 2024;25:bbae364. https://doi.org/10.1093/bib/bbae364

129. Alobaidi S. Emerging biomarkers and advanced diagnostics in chronic kidney disease: early detection through multi-omics and AI. *Diagnostics (Basel)*. 2025;15:1225. https://doi.org/10.3390/diagnostics15101225

130. de Souza VBC, Jordan BT, Tseng E, et al. Transformation of alignment files improves performance of variant callers for long-read RNA sequencing data. *Genome Biol*. 2023;24:91. https://doi.org/10.1186/s13059-023-02923-y

131. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: global dimension and perspectives. *Lancet*. 2013;382:260–272. https://doi.org/10.1016/S0140-6736(13)60687-X

132. Franceschini N, Feldman DL, Berg JS, et al. Advancing genetic testing in kidney diseases: report from a national kidney foundation working group. *Am J Kidney Dis*. 2024;84:751–766. https://doi.org/10.1053/j.ajkd.2024.05.010

133. Cusick MM, Tisdale RL, Adams AS, et al. Balancing efficiency and equity in population-wide CKD screening. *JAMA Netw Open*. 2025;8:e254740. https://doi.org/10.1001/jamanetworkopen.2025.4740

134. Shafin K, Pesout T, Chang PC, et al. Haplotype-aware variant calling with PEPPER-Margin-DeepVariant enables high accuracy in nanopore long-reads. *Nat Methods*. 2021;18:1322–1332. https://doi.org/10.1038/s41592-021-01299-w

135. Zook JM, McDaniel J, Olson ND, et al. An open resource for accurately benchmarking small variant and reference calls. *Nat Biotechnol*. 2019;37:561–566. https://doi.org/10.1038/s41587-019-0074-6

136. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. *Nat Rev Genet*. 2016;17:333–351. https://doi.org/10.1038/nrg.2016.49

137. Quick J, Loman NJ, Duraffour S, et al. Real-time, portable genome sequencing for Ebola surveillance. *Nature*. 2016;530:228–232. https://doi.org/10.1038/nature16996

138. De Coster W, De Rijk P, De Roeck A, et al. Structural variants identified by Oxford nanopore PromethION sequencing of the human genome. *Genome Res*. 2019;29:1178–1187. https://doi.org/10.1101/gr.244939.118

139. Sedlazeck FJ, Rescheneder P, Smolka M, et al. Accurate detection of complex structural variations using single-molecule sequencing. *Nat Methods*. 2018;15:461–468. https://doi.org/10.1038/s41592-018-0001-7

140. Simpson JT, Workman R, Zuzarte PC, David M, Dursi LJ, Timp W. 2016.

141. Liu Y, Rosikiewicz W, Pan Z, et al. DNA methylation-calling tools for Oxford nanopore sequencing: a survey and human epigenome-wide evaluation. *Genome Biol*. 2021;22:295. https://doi.org/10.1186/s13059-021-02510-z

142. Jain M, Koren S, Miga KH, et al. Nanopore sequencing and assembly of a human genome with ultra-long reads. *Nat Biotechnol*. 2018;36:338–345. https://doi.org/10.1038/nbt.4060

143. De Coster W, Weissensteiner MH, Sedlazeck FJ. Towards population-scale long-read sequencing. *Nat Rev Genet*. 2021;22:572–587. https://doi.org/10.1038/s41576-021-00367-3

144. Ni P, Nie F, Zhong Z, et al. DNA 5-methylcytosine detection and methylation phasing using PacBio circular consensus sequencing. *Nat Commun*. 2023;14:4054. https://doi.org/10.1038/s41467-023-39784-9