Table 1 | **Major genetic testing modalities: indications and limitations**

| Modality | Scope | Indications for use | Examples | Advantages | Disadvantages |
|---|---|---|---|---|---|
| Sanger sequencing | Detection of SNVs and small indels (<10 bp) within a DNA segment of <1 kb | • Confirmation of NGS findings<br>• Regions refractory to NGS, such as GC-rich, highly repetitive segments<br>• Patients whose phenotype is indicative of a disorder caused by mutations in one specific gene | • Confirm frameshift *COL4A3* variant detected by NGS<br>• Diagnostic testing for Fabry disease<br>• Detect *CTNS* mutation (nephropathic cystinosis) in a patient with corneal cystine crystals and Fanconi syndrome | • High analytical accuracy<br>• Easier and faster sequence interpretation compared with multigene testing enables faster turnaround time<br>• No risk of secondary findings | • Resolution <1 kb; cannot detect larger structural variants<br>• Increasingly time- and cost-inefficient with increasing gene length and/or number of genes tested |
| Chromosomal microarray | Genome-wide detection of CNVs ≥200–400 kb | Patients with phenotypes commonly resulting from genomic imbalances, such as multiple congenital anomalies | • Detect whole-gene deletion of *HNF1B* in a patient with renal hypodysplasia and autism<br>• Detect 22q11.2 deletion (DiGeorge syndrome) in a patient with renal agenesis and neonatal hypocalcaemia | • Higher resolution enables detection of CNVs missed by karyotyping<br>• Genome-wide CNV detection increases diagnostic sensitivity | • Cannot detect SNVs, indels, and small CNVs<br>• Limited ability to detect balanced chromosomal rearrangements, low-grade somatic mosaicism, and CNVs in certain regions (such as pseudogenes and repetitive elements) |
| Targeted NGS panels | Detection of SNVs and small indels (<1 kb) within genes of interest for the clinically suspected phenotype | • Patients with phenotypes that are fairly specific for a particular disorder<br>• Disorders with low genetic and/or phenotypic heterogeneity | • Testing *AGXT*, *HOGA1*, and *GRHPR* for primary hyperoxaluria in a patient with childhood-onset calcium oxalate urolithiasis<br>• Testing for *COL4A3*, *COL4A4*, and *COL4A5* mutations in a patient with suspected Alport syndrome | • Can be optimized to ensure sufficient coverage of variants in targeted regions<br>• Interrogation of genes that are related to the clinical indication facilitates interpretation and minimizes risk of secondary findings | • Testing a limited number of genes decreases diagnostic sensitivity, especially for genetically and/or phenotypically heterogeneous disorders<br>• Challenges of panel design (gene selection and need for frequent updates)<br>• Minimal capacity for sequence reanalysis |
| Whole-exome sequencing | Detection of SNVs and small indels (<1 kb) within coding regions of the genome | • Patients with highly genetically heterogeneous or nonspecific phenotypes<br>• CKD of unknown aetiology<br>• Patients left undiagnosed by targeted NGS panels | • NPHP-RC[122,123]<br>• Diagnosis of congenital chloride diarrhoea in an unresolved case of presumed Bartter syndrome[148]<br>• Diagnosis of *LMX1B* glomerulopathy in familial ESRD of unknown origin[165] | • Unbiased approach increases diagnostic sensitivity<br>• Interrogation of the coding regions that are enriched for known disease-causing mutations is a cost-effective approach to genome-wide testing<br>• Genome-wide scope enables sequence reanalysis and discovery of novel genes | • Lower analytical sensitivity and specificity than whole-genome sequencing owing to limited coverage of certain regions and inability to accurately call certain types of variants (such as indels)<br>• Approach can lead to multiple candidate variants, increasing time required for interpretation and need for follow-up testing<br>• Burden of secondary findings in genes unrelated to the primary indication for testing |
| Whole-genome sequencing | Detection of SNVs and small indels (<1 kb) within coding and non-coding regions of the genome | • Patients with highly genetically heterogeneous phenotypes<br>• Patients with nonspecific phenotypes<br>• CKD of unknown aetiology<br>• Patients left undiagnosed by all other genetic testing modalities | • Detection of causal intronic variants, for example, in a genetically unresolved case of Gitelman syndrome[106]<br>• Genetic diagnosis of ADPKD (owing to high sequence homology of *PKD1*)[110]<br>• Detection of causal balanced translocations for congenital anomalies[294,295] | Superior diagnostic and analytical sensitivity to whole-exome sequencing owing to its ability to assess SNVs, indels, and CNVs in coding and non-coding regions and more complete per-base coverage | • Difficulty of interpreting non-coding variants<br>• Large amount of data generated results in substantial time and monetary costs, hindering return of results<br>• Burden of secondary findings in genes unrelated to the primary indication for testing<br>• Burden of long-term sequence data storage |

ADPKD, autosomal dominant polycystic kidney disease; ADTKD-*MUC1*, autosomal dominant tubulointerstitial kidney disease due to mutations in *MUC1*; *AGXT*, alanine-glyoxylate aminotransferase; CKD, chronic kidney disease; CNV, copy number variant; COL4A, collagen type IV α-chain; *CTNS*, cytinosin, lysosomal cystine transporter; ESRD, end-stage renal disease; GC, guanine-cytosine; *GRHPR*, glyoxylate and hydroxypyruvate reductase; *HOGA1*, 4-hydroxy-2-oxoglutarate aldolase 1; *HNF1B*, HNF1 homeobox B; *LMX1B*, LIM homeobox transcription factor 1β; *MUC1*, mucin 1, cell surface associated; NGS, next-generation sequencing; NPHP-RC, nephronophthisis-related ciliopathy; *PKD1*, polycystin 1, transient receptor potential channel interacting; SNVs, single-nucleotide variants.