REVIEW

Applications of genome sequencing as a single platform for clinical constitutional genetic testing

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ABSTRACT

The number of human disease genes has dramatically increased over the past decade, largely fueled by ongoing advances in sequencing technologies. In parallel, the number of available clinical genetic tests has also increased, including the utilization of exome sequencing for undiagnosed diseases. Although most clinical sequencing tests have been centered on enrichment-based multigene panels and exome sequencing, the continued improvements in performance and throughput of genome sequencing suggest that this technology is emerging as a potential platform for routine clinical genetic testing. A notable advantage is a single workflow with the opportunity to reflexively interrogate content as clinically indicated; however, challenges with implementing routine clinical genome sequencing still remain. This review is centered on evaluating the applications of genome sequencing as a single platform for clinical constitutional genetic testing, including its potential utility for diagnostic testing, carrier screening, cytogenomic molecular karyotyping, prenatal testing, mitochondrial genome interrogation, and pharmacogenomic and polygenic risk score testing.

Introduction

High-throughput sequencing is a widely used technology for clinical constitutional genetic testing, which is commonly implemented as enrichment-based multigene panels for diagnostic testing,[1] carrier screening,[2] and enrichment-based exome sequencing for undiagnosed disease.[3,4] However, genome sequencing is increasingly being considered as a potential platform for clinical testing at some institutions and laboratories.[5,6] In addition to the increased likelihood of Mendelian disease diagnosis, the operational advantages

with clinical genome sequencing include a single laboratory workflow and the opportunity to reflexively interrogate additional clinical content when clinically indicated.[7-9] However, several challenges with implementing routine clinical genome sequencing still remain, including cost-effectiveness, computational infrastructure, and both logistical and ethical considerations.

Genome sequencing has technical advantages over enrichment-based sequencing, including less bias and more consistent coverage across coding regions,[10] as well as improved capacity to detect copy-number variants (CNVs) and

breakpoints,[11] and ongoing improvements in repeat expansion detection.[12] Moreover, genome sequencing inherently has advantages with interrogating noncoding variants for both gene/variant discovery and clinical testing,[13] and computational algorithms are continually improving to facilitate the interrogation of challenging homologous regions[14] and repeat expansions.[15] Data have also emerged indicating that genome sequencing has a higher diagnostic yield compared with exome sequencing in pediatric and other clinical service lines,[16-18] including rapid genome sequencing for critically ill children.[19] Although the vast majority of clinical genome sequencing currently uses short-read sequencing platforms, long-read sequencing platforms continue to improve in performance and throughput, which has translated to higher variant calling accuracy in historically challenging genomic contexts.[20]

As the accessibility of genome sequencing for clinical laboratories continues to improve, resources and regulatory recommendations that support clinical genome sequencing are continually evolving, including design recommendations, analytical validation resources, and professional guideline statements (Table 1[21-46]). This review is centered on evaluating the applications of genome sequencing for routine clinical constitutional genetic testing, including its utility as a technology for diagnostic testing, carrier screening, cytogenomic molecular karyotyping, prenatal testing, mitochondrial genome interrogation, and pharmacogenomic and polygenic risk score testing. Of note, ethics evaluations and thorough cost-effectiveness analyses of clinical genome sequencing, as well as prenatal cell-free DNA screening, were considered outside the scope of this review.

Table 1   Resources for clinical genome sequencing development and validation

Category	Organization/Entity	Year	Resource	Reference
Professional Guidelines				
	CDC	2012	Clinical sequencing quality recommendations	Gargis et al[21]
	AMP	2012	Clinical genome sequencing recommendations	Schrijver et al[22]
	ACMG	2013	Clinical sequencing laboratory standards	Rehm et al[23]
	CAP	2014	Clinical sequencing proficiency standards	Schrijver et al[24]
	CAP	2015	Clinical sequencing laboratory standards	Aziz et al[25]
	CDC	2015	Clinical sequencing informatics best practices	Gargis et al[26]
	CCMG	2015	Clinical genome sequencing for monogenic disease statement	Boycott et al[27]
	ESHG	2016	Clinical sequencing guidelines	Matthijs et al[28]
	CAP	2017	Clinical sequencing development and validation recommendations	Santani et al[29]
	CAP	2017	Clinical exome/genome sequencing development and validation recommendations	Hegde et al[30]
	AMP/CAP	2018	Clinical sequencing bioinformatic validation guidelines	Roy et al[31]
	CAP	2019	Clinical sequencing design and implementation	Santani et al[32]
	CCMG	2019	Clinical sequencing laboratory guidelines	Hume et al[33]
	ACMG	2020	Clinical sequencing test development guidelines	Bean et al[34]
	MGI	2020	Clinical genome sequencing validation recommendations	Marshall et al[35]
	MGI	2020	Clinical genome sequencing utility	Hayeems et al[36]
	ACMG	2021	Clinical exome and genome sequencing guideline	Manickam et al[37]
	MGI	2022	Clinical genome sequencing interpretation and reporting	Austin-Tse et al[38]
	ESHG	2022	Clinical genome sequencing implementation recommendations	Souche et al[39]
	CLSI	2023	Cinical laboratory sequencing standards and recommendations	CLSI[47]
Analytical Validation and Reference Materials				
	GIAB/NIST	2016	Genome sequencing benchmarking reference material	Zook et al[40]
	GIAB/NIST	2018	Reference material benchmarking best practices	Cleveland et al[41]
	GIAB/NIST	2019	Small-variant benchmarking reference material	Zook et al[42]
	GA4GH	2019	Small-variant benchmarking best practices	Krusche et al[43]
	GIAB/NIST	2020	Deletion/insertion benchmarking reference material	Zook et al[44]
	ClinGen/GeT-RM	2021	Clinical genome sequencing in silico reference material	Wilcox et al[45]
	GIAB/NIST	2022	Benchmarking reference material for challenging medically relevant genes	Wagner et al[46]

ACMG, American College of Medical Genetics and Genomics; *AMP*, Association for Molecular Pathology; *CAP*, College of American Pathologists; *CCMG*, Canadian College of Medical Geneticists; *CDC*, Centers for Disease Control and Prevention; *ClinGen*, Clinical Genome Resource; *CLSI*, Clinical and Laboratory Standards Institute; *ESHG*, European Society of Human Genetics; *GA4GH*, Global Alliance for Genomics and Health; *GeT-RM*, Genetic Testing Reference Materials Coordination Program; *GIAB*, Genome in a Bottle Consortium; *MGI*, Medical Genome Initiative; *NIST*, National Institute of Standards and Technology.

Clinical constitutional genome sequencing

Short- and long-read genome sequencing

Short-read sequencing is the most commonly implemented platform for genome sequencing, which is driven by its throughput and cost-effectiveness, as well as the accessibility of the platform infrastructure. Clinical constitutional short-read genome sequencing applications typically require an average autosomal depth of >30 or 40× (Figure 1)[35,48,49]; however, low-pass (~0.5-5×) short-read sequencing has also been used for germline copy-number profiling, as well as small-variant genotyping when coupled with imputation. However, long-read genome sequencing has recently emerged as an important alternative,[18,50,51] based on its improved interrogation of clinically significant genomic regions (Figure 1[48,49]), including expansions, structural variation, homologous regions, and the human leukocyte antigen (HLA) locus, as well as its capacity for variant phasing.[52,53] Both single-molecule real-time (SMRT; HiFi; Pacific Biosciences) and nanopore (Oxford Nanopore Technologies) long-read genome sequencing have been used for constitutional variant detection applications using both high-depth and low-pass sequencing.[54-57] As such, future iterations of clinical genome sequencing could be developed as 2 parallel workflows to strategically leverage the benefits of either high-depth or low-pass sequencing depending on the intended use and economics of the application.

Clinical genome sequencing resources

Although sequencing depth is largely considered the most critical quality metric for clinical genome sequencing, other quality metrics and thresholds are necessary to establish and monitor when developing a clinical genome sequencing assay (eg, callability and mappability).[35] Importantly,
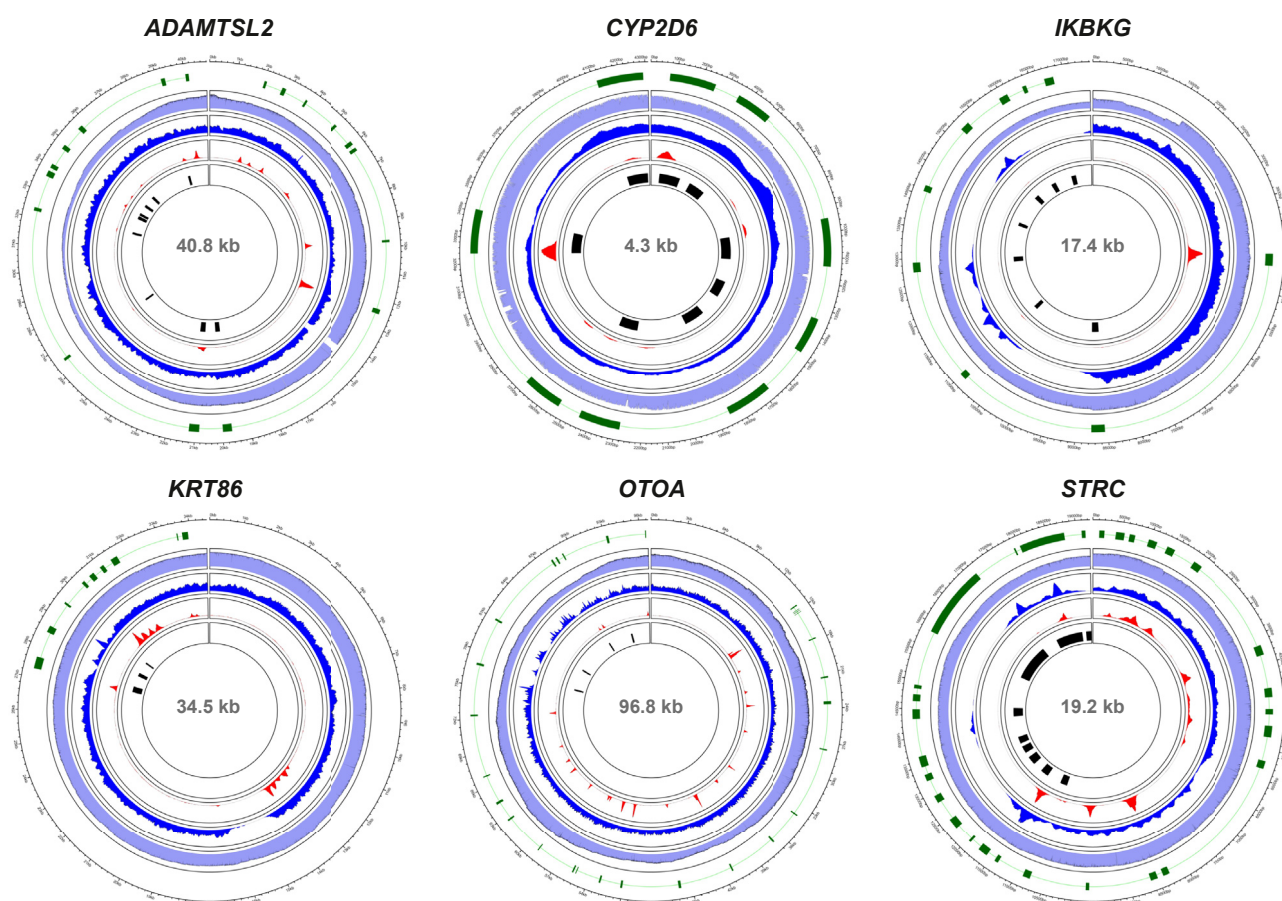

Figure 1   Illustration of germline genome architecture and sequencing accessibility, as represented by 6 clinically significant genes. From top left to bottom right: *ADAMTSL2* (NM_014694.4; geleophysic dysplasia), *CYP2D6* (NM_000106.6; drug metabolism), *IKBKG* (NM_001099857.5; incontinentia pigmenti), *KRT86* (NM_001320198.2; monilethrix), *OTOA* (NM_144672.4; sensorineural hearing loss), and *STRC* (NM_153700.2; sensorineural hearing loss) (GRCh38). From inner to outer circles: previously reported exon-level sequencing "dead zones" across the genome (lifted over to GRCh38 from GRCh37; black)[48]; enrichment-based short-read exome sequencing coverage (average 185.8× across all coding regions; red); short-read genome sequencing coverage (average 33.2× across all coding regions; dark blue); long-read HiFi genome sequencing coverage (average 27.5× across all coding regions; light blue); and gene transcripts (introns: light green; exons: dark green). Noncoding exons were excluded from transcript tracks. All sequencing data were acquired from publicly available GIAB/NIST reference material sample NA12878 (HG001), filtered with mapping and base quality scores greater than Q20, and Circos images generated using R package circlize.[49]

support for the implementation of clinical genome sequencing is currently available from several venues (Table 1[21-46]), including guidelines from the College of American Pathologists (CAP),[25] the American College of Medical Genetics and Genomics (ACMG),[37] the Association for Molecular Pathology (AMP),[31] and related professional consortia[21,29,32,58]; benchmarking reference materials from the Genome in a Bottle (GIAB)/National Institute of Standards and Technology (NIST) consortium,[42] the Global Alliance for Genomics and Health consortium,[43] the Coriell Cell Repository/CDC Genetic Testing Reference Materials Coordination Program[59]; and clinical genome sequencing recommendations from the Medical Genome Initiative.[35,36,38] The expansion of available resources, including large publicly available genomic databases that support variant interpretation (eg, The Genome Aggregation Database[60] and ClinVar[61]) and classification[62] (Table 2[60-76]),

suggest that clinical laboratories will continue to adopt genomic-based diagnostic platforms in the future.

Clinical genome sequencing applications

Diagnostic panels and exome sequencing

High-throughput sequencing of targeted gene panels and exome sequencing has resulted in a dramatic improvement over the traditional diagnostic pathway, which often included a prolonged diagnostic odyssey due to low diagnostic yields from sequential gene testing for Mendelian diseases. The first demonstrated use of exome sequencing for the diagnosis of a rare genetic condition of unknown etiology was reported in 2010,[77] which prompted a series of publications on the utility of targeted panels and exome sequencing for the diagnosis of pediatric and adult patients with genetic diseases. In parallel to the last decade of

Table 2 Resources for clinical genome sequencing variant interpretation

Category	Organization/Entity	Year	Resource	Reference
Germline Sequence Variants				
	HGMD	2003	Collection of published germline variants implicated in human inherited disease	Stenson et al[63]
	AMP/ACMG	2015	Standards and guidelines for the interpretation of sequence variants	Richards et al[62]
	ClinGen	2015	SVI Working Group recommendations on using the ACMG/AMP guidelines	Rehm et al[64]
	ClinGen	2015	SVI Expert-Panel-specified ACMG/AMP variant interpretation guidelines	Rehm et al[64]
	ClinVar	2016	Database of genetic variation and clinical interpretation	Landrum et al[61]
	gnomAD	2017	Aggregated exome and genome sequencing population database	Karczewski et al[60]
	CPIC	2017	Standardized pharmacogenomic allele function and phenotype terms	Caudle et al[65]
	OMIM	2019	Database of human genes and genetic disorders	Amberger et al[66]
	UK-ACGS	2020	Practice guidelines for variant classification in rare disease	Ellard et al[67]
	PharmGKB	2021	Comprehensive resource of pharmacogenomic variation and knowledge	Whirl-Carrillo et al[68]
	TOPMed	2021	Aggregated genome sequencing population database	Taliun et al[69]
	GenCC	2022	Mendelian disease gene curation database	DiStefano et al[70]
Germline Structural Variants				
	DECIPHER	2009	Interactive web-based database of human genomic variation and phenotypes	Firth et al[71]
	DGV	2014	Curated collection of structural variations in the human genome	MacDonald et al[72]
	ClinGen	2018	Human gene dosage sensitivity map	Riggs et al[73]
	ACMG/ClinGen	2020	Technical standards for the interpretation and reporting of constitutional CNVs	Riggs et al[74]
Mitochondrial Variants				
	MitoMap	1996	Compendium of variants in human mtDNA	Kogelnik et al[75]
	ClinGen (mtDNA)	2020	Adapted ACMG/AMP standards and guidelines for mtDNA variant interpretation	McCormick et al[76]

ACGS, Association for Clinical Genomic Science; *ACMG*, American College of Medical Genetics and Genomics; *AMP*, Association for Molecular Pathology; *ClinGen*, Clinical Genome Resource; *CPIC*, Clinical Pharmacogenetics Implementation Consortium; *DGV*, Database of Genomic Variants; *GenCC*, Gene Curation Coalition; *gnomAD*, Genome Aggregation Database; *HGMD*, Human Gene Mutation Database; *OMIM*, Online Mendelian Inheritance in Man; *PharmGKB*, Pharmacogenomics Knowledgebase; *TOPMed*, Trans-Omics for Precision Medicine.

high-throughput sequencing-based gene discovery research, professional societies (eg, ACMG and AMP), and regulatory agencies (eg, CAP) have published important clinical guidelines on the use of high-throughput sequencing in the diagnostic setting (Table 1[21-46]). Additionally, selected societies and federally funded consortia dedicated to gene and variant curation (eg, Clinical Genome Resource [ClinGen][64] and Gene Curation Coalition[70]) have emerged as central resources for diagnostic testing implementation and variant interpretation (Table 2[60-76]).

Diagnostic constitutional sequencing is commonly implemented as multigene enrichment-based panels for specific clinical indications, which have the advantage of being optimized for quality and depth of coverage across targeted regions of interest. However, technical limitations include restricted updates to panel content, variant interpretation limited to coding regions (unless specifically targeted), and reduced sensitivity for detecting CNV breakpoints (and single exon copy-number changes).[78] In contrast, enrichment-based exome sequencing aims to capture all protein-coding exons representing ~2% of the genome. In addition, exome sequencing is often implemented as family or trio based, which can facilitate proband variant interpretation under autosomal dominant, recessive, and/or X-linked inheritance models.[34] As the cost of sequencing has decreased, exome sequencing has been rapidly adopted as a first-tier test for undiagnosed conditions, particularly among patients with syndromic indications or for whom traditional diagnostic modalities have been uninformative. However, although exome sequencing is considered to have strong diagnostic utility, its overall diagnostic yield remains at ~25% to 30% across indications (eg, neurodevelopmental disorders, multiple congenital anomalies, and congenital heart disease).[3,79,80] Limitations of exome sequencing include the inability to detect disease-associated intronic variants if not specifically targeted, as well as some coding variants in difficult regions. An increasingly utilized application of exome sequencing among clinical laboratories is an "in silico enrichment exome" approach (ie, "exome slice") that leverages a single underlying enrichment-based exome platform and laboratory workflow but bioinformatically restricts the clinical interpretation to specific diagnostic gene panel regions of interest.[81-83]

In contrast to enrichment-based exome sequencing, genome sequencing evaluates approximately 95% of nuclear and mitochondrial DNA, and it is increasingly being considered for diagnostic constitutional genetic testing.[84] Diagnostic yield comparisons between genome and exome sequencing are increasingly being reported, which suggest an improvement that may be influenced by age of presentation and clinical indication.[16-18,85,86] Moreover, rapid genome sequencing has become feasible for the diagnosis of critically ill newborns presenting with suspected genetic conditions in the NICU,[87] and some national health care systems are currently implementing genome sequencing broadly to guide medical management among seriously ill children.[88] Consistent with the "exome slice" approach, genome-based diagnostic panels ("genome slice") can also be developed by clinical laboratories when implementing genome sequencing as a single workflow. This approach inherently has the added benefit of offering flexibility in additional reporting content for a variety of Mendelian diagnostic panels to guide patient care, clinical management, prognosis, and disease risk screening as needed (Figure 2[49]). Moreover, as clinical RNA sequencing increasingly becomes available to resolve the functional significance of germline variants, the interrogation of noncoding variants by genome sequencing will likely have continued increasing value for diagnostic testing.[89-91] However, this flexibility in reporting content is offset by the necessary computational infrastructure needed from clinical laboratories to develop, validate, and maintain a clinical genome sequencing workflow.

Genome sequencing and carrier screening

In contrast to diagnostic genomic testing, carrier screening tests clinically asymptomatic individuals for pathogenic variants associated with autosomal recessive and X-linked disorders for the purpose of reproductive risk management.[2,92] The American College of Obstetricians and Gynecologists (ACOG) recommends screening be offered to all pregnant women,[92,93] ideally coupled with genetic counseling and performed preconception so that carrier couples can understand their reproductive risks and make informed decisions. ACOG currently recommends screening for cystic fibrosis, spinal muscular atrophy, and hemoglobinopathy for all pregnant women regardless of ancestry or ethnicity.[14,92,94] However, screening for a limited number of additional recessive conditions is also recommended for reproductive women of certain ethnicities and/or those with a family history of a particular genetic disorder (eg, Tay-Sachs disease and fragile X syndrome).[92,95] The ACMG has recently reported updated guidance on screening, which recommends a tiered system for panel gene content based on heterozygote frequency, among other important considerations related to implementation and result management.[2]

Screening technologies have evolved from targeted genotyping of pathogenic variants to high-throughput full-gene sequencing, as well as orthogonal copy-number techniques for selected recessive genes with recurrent deletion alleles. As such, pan-ethnic expanded screening can now test for hundreds of conditions simultaneously, which is more efficient and cost-effective than targeted screening. Although ACOG indicates that expanded screening is an acceptable strategy for prepregnancy and prenatal screening, a heterozygote frequency threshold of 1 in 100 has been recommended when incorporating genes/diseases into test panels to minimize patient anxiety as more heterozygotes are identified.[93] As such, there is ongoing debate around the utility and rationale of expanded screening panels,[96,97] as currently available commercial panels often include recessive conditions with low-frequency heterozygote disease alleles. However, these expanded panels with lower
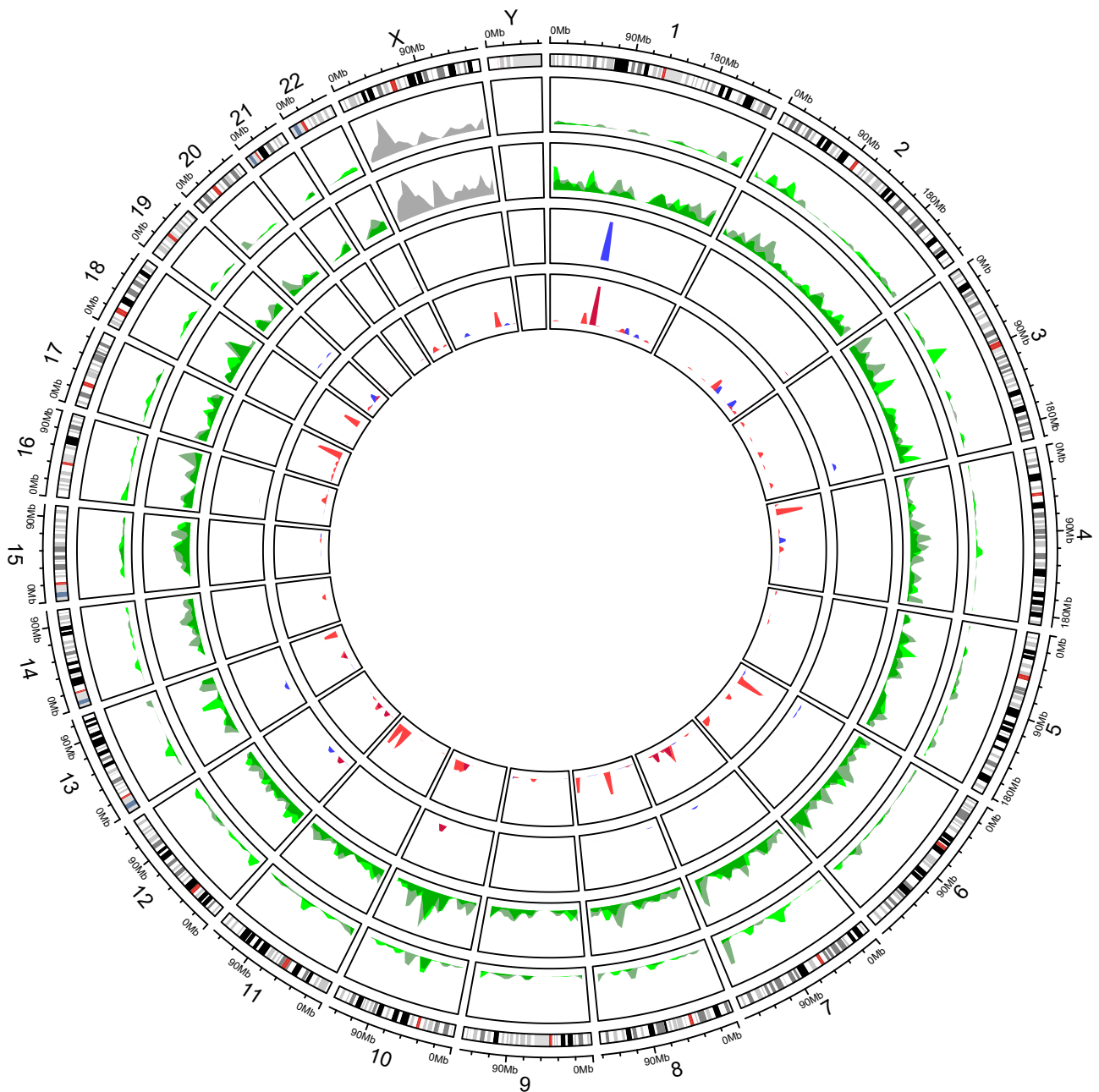
Figure 2   Illustration of Mendelian disease genes and pharmacogenomic genes across the human genome (GRCh38). From inner to outer circles: genes defined by the US FDA Table of Pharmacogenetic Associations as having "supportive evidence for therapeutic management recommendations" (blue) and "potentially impacted"/ "pharmacokinetics only" (red); genes with CPIC evidence levels A, A/B, B (blue) and C, C/D, D (red); OMIM-defined recessive (light green), dominant (dark green), and X-linked (gray) disease genes with an established molecular etiology (phenotype mapping key: 3); and ClinGen-defined recessive (light green), dominant (dark green), and X-linked (gray) disease genes with "definitive" or "strong" evidence. All data were downloaded from primary sources and Circos images generated using R package circlize.[49]

heterozygote frequencies are supported by recently reported data indicating that the recommended 1 in 100 carrier frequency threshold can lead to gene panels with significantly reduced detection of at-risk couples,[98] particularly when considering multiethnic populations.[99]

Genome sequencing has potential utility for screening, including improved detection rates for certain conditions and the flexibility to interrogate additional disease genes or genomic regions when indicated. Of note, some recessive conditions with recurrent pathogenic CNV alleles (eg, spinal muscular atrophy) have variable detection rates when screened solely by enrichment-based sequencing panels without ancillary methods; however, bioinformatic tools have recently been developed that detect *SMN1* and *SMN2* deletion heterozygotes from enrichment-based targeted sequencing[100] and genome sequencing with high

accuracy.[14] Preconception screening for 728 gene-disease pairs using genome sequencing has also recently been reported, which resulted in improved sensitivity compared with targeted screening for clinically significant variant detection, including CNVs and noncoding variants.[101] Importantly, genome sequencing can theoretically identify carrier status across 3104 autosomal and X-linked recessive genes in the human genome, as defined by OMIM[66] (Figure 2[49]). This enhanced capability of disease gene inclusion by genome sequencing is counterbalanced by the ongoing debate over the clinical utility of expanded screening panels; however, to facilitate more informed and compartmentalized gene/disease panel development, a taxonomy framework has been proposed that categorizes potential gene content into lifespan limiting, serious, mild, unpredictable, and adult-onset,[102] with the overarching aim of minimizing anxiety when detecting more heterozygotes for very rare and/or less severe conditions.

Genome sequencing and cytogenomic molecular karyotyping

Cytogenetic testing is arguably the original genomic platform, which interrogates chromosomal variation through metaphase cell preparations and microscopy-based karyotyping. Aneuploidies, large structural rearrangements (eg, translocations and inversions), and polyploidy can be detected by traditional cytogenetic testing; however, copy-number aberrations less than ~5 Mb are below the limit of detection for high-resolution chromosome analyses. This prompted the implementation of clinical fluorescence in situ hybridization testing to interrogate recurrent constitutional deletions and duplications less than 5 Mb, as well as genome-wide chromosomal microarray (CMA) testing with resolutions defined by the density of oligonucleotide probes. CMA is now a common first-tier platform for copy-number testing across several clinical indications, and resolution typically ranges from ~10 to 100 kb depending on CMA design and platform. Critical resources for CNV interpretation include the Database of Genomic Variants (DGV), which catalogs structural variation (>50 bp) in healthy population cohorts[72]; the ClinGen Dosage Sensitivity Map[73] and DECIPHER,[71] which catalog genomic variation in patient cohorts (Figure 3[49]); and quantitative CNV classification recommendations have been reported by the ACMG (Table 2).[60-76] In addition, the Genome Aggregation Database has increasingly incorporated structural variants identified by sequencing, including deletions, duplications, CNVs, insertions, inversions, and other variant types.[103]

The implementation of constitutional CMA testing as an orthogonal platform for chromosome analysis has evolved the field of cytogenetics from low-resolution karyotyping to high-resolution molecular karyotyping. In addition to now being a routine clinical genetic test, CMA profiling of both patient population and general population cohorts has resulted in the discovery of significant copy-number variability across the human genome that previously was not appreciated, including a spectrum of benign, uncertain, pathogenic, and susceptibility CNVs. However, technical limitations of CMA testing include the inability to precisely identify CNV breakpoints and the genomic location(s) of copy-number gain material. Consistent with the ongoing improvements in both short- and long-read sequencing chemistries and throughput, bioinformatic algorithms that leverage local sequencing depth and/or read orientation have now enabled the identification of constitutional CNVs and other structural rearrangements by sequencing.

Enrichment-based diagnostic sequencing panels now routinely include CNV detection; however, given that the sequencing reads in these panels are localized to coding regions, precise reporting of CNV breakpoints is technically not possible. As such, the uniform coverage of genome sequencing provides an immediate improvement in CNV detection and breakpoint localization compared with enrichment-based sequencing. Many bioinformatic tools for constitutional copy-number detection from short-read sequencing data are currently available,[104] as are long-read sequencing structural variant callers for both HiFi and nanopore sequencing.[105] Importantly, analytical validation of CNV detection by genome sequencing can now be accomplished, in part, using the NA24385 (HG002) GIAB/NIST reference sample with a reported structural variant benchmarking truth set (Table 1).[21-46]

Notably, low-pass (~5×) genome sequencing has been reported to have high accuracy and precision for clinically significant CNV detection compared with CMA analysis, which included enhanced sensitivity and cost-effectiveness,[106] and the detection of absence of heterozygosity.[107] Moreover, both short- and long-read genome sequencing are increasingly emerging as efficient tools for translocation breakpoint detection,[108-110] underscoring the potential utility of genome sequencing for more comprehensive molecular karyotyping. Given the ongoing improvements and resources supporting CNV and structural variant detection by genome sequencing, molecular karyotyping by clinical genome sequencing platforms will likely supplant CMA and/or karyotyping in the future when workflow feasibility is justified.

Prenatal genome sequencing

Prenatal genetic testing has unique characteristics compared with postnatal constitutional testing because the specimens are procured from invasive procedures during pregnancy, including chorionic villus sampling and amniocentesis. Given the inherent fetal risk of these procedures and the sensitivities around prenatal genetic result interpretation and counseling, prenatal testing has historically been limited to targeted variant testing when parents are positive for a pathogenic variant, or sequencing panels, karyotyping, fluorescence in situ hybridization, and/or CMA testing for fetuses with abnormal ultrasound findings. The ACOG recommends CMA testing to any patient undergoing invasive diagnostic testing, as well as being the primary test for pregnancies with a fetal structural abnormality detected
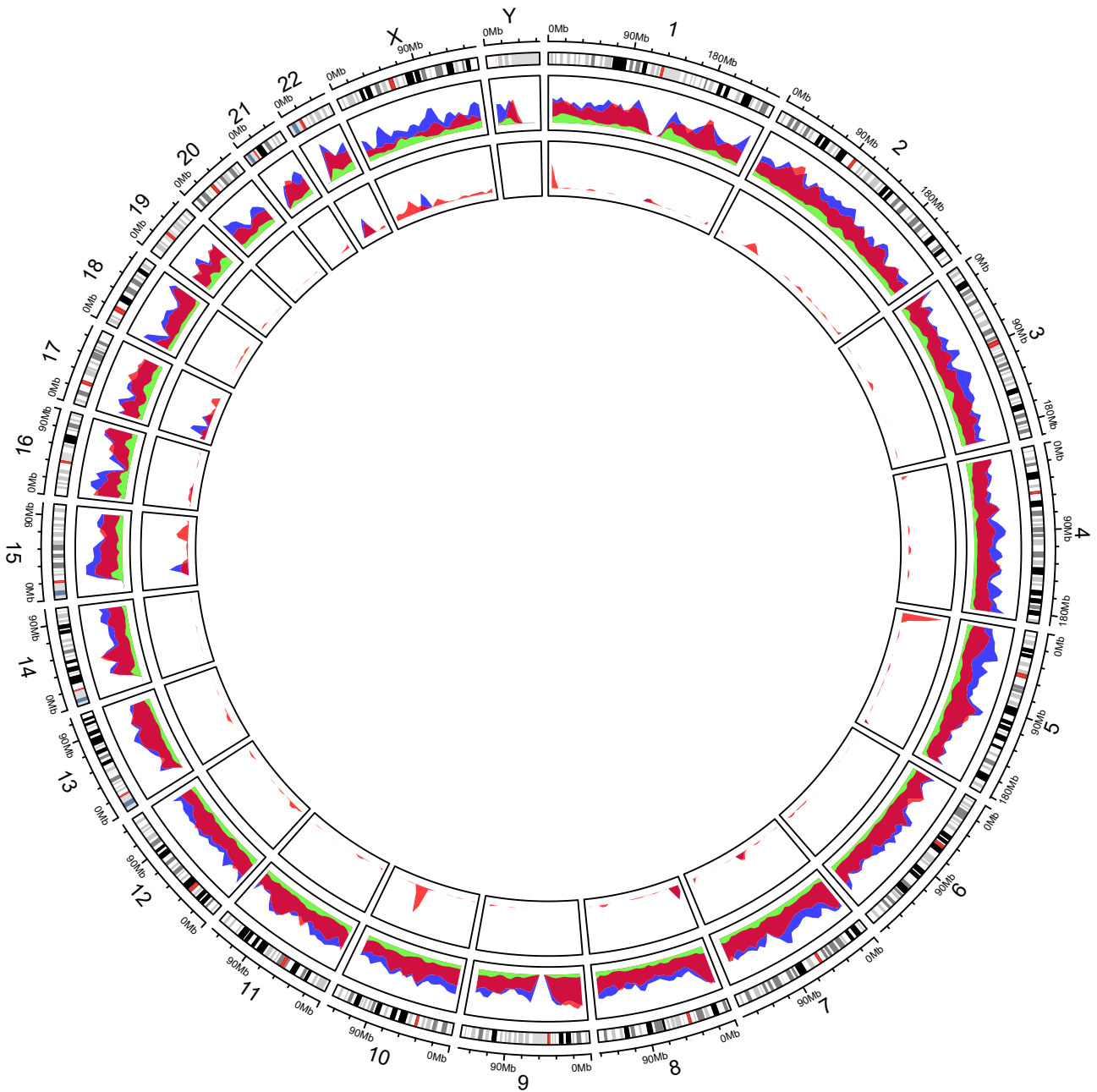
Figure 3   Illustration of structural variation across the human genome (GRCh38). From inner to outer circles: ClinGen genes and genomic regions with "sufficient" or "emerging" evidence curated as haploinsufficient (red) and triplosensitive (blue) and DGV Gold Standard deletions (red), duplications (blue), and "other" structural variants (as defined by DGV; light green) found in the general population. All data were downloaded from primary sources and Circos images generated using R package circlize.[49]

by ultrasound,[111] including a recommendation "against" routine prenatal exome or genome sequencing.[112] However, ACOG states that "prenatal exome sequencing may be reasonable for fetuses with multiple anomalies or in cases of recurrent fetal phenotypes with no diagnosis by karyotype or CMA."[112] Similarly, the ACMG recommends "considering exome sequencing only when specific genetic tests for a phenotype, including targeted sequencing, have failed to determine a diagnosis in a fetus with multiple congenital anomalies suggestive of a genetic disorder."[113]

Despite the increased diagnostic rate of exome sequencing compared with targeted testing, a limitation for prenatal exome testing is the longer turnaround time, which can negatively affect reproductive decision making. Another major limitation is the higher number of variants of uncertain significance, which can be challenging for providers and patients to interpret, as well as lead to increased patient anxiety. However, rapid prenatal exome testing is currently available, and evidence is continuing to emerge, indicating a high diagnostic yield for fetuses with abnormal ultrasound

findings (increased nuchal translucency, hydrops, intrauterine growth restriction, and/or congenital anomalies).[114,115] As such, it is likely that as prenatal exome testing workflows become more efficient, including variant filtration and prioritization algorithms, that the relevant professional societies will report more supportive recommendations for prenatal exome testing under certain clinical contexts.

Less data are available on constitutional prenatal genome sequencing, which is also not commonly implemented clinically. However, there is potential with implementing genome sequencing prenatally because both coding and noncoding pathogenic variants can be interrogated, as well as the improved capacity to detect CNVs and balanced chromosomal abnormalities. This is counterbalanced by the understandable concerns about identifying more information than needed through prenatal genome sequencing, including more variants of uncertain significance, late-onset disease variants and susceptibility alleles, which could overwhelm providers, counselors, and patients.[116] Similar to postnatal molecular karyotyping, the potential utility of prenatal genome sequencing is supported by the prenatal discovery of a balanced translocation that disrupted the CHD7 gene, which confirmed a CHARGE syndrome diagnosis,[117] and subsequent cohort studies on additional prenatal subjects with apparently balanced chromosomal rearrangements and normal CMA results.[118] More recently, low-pass (<1×) genome sequencing among 1023 women undergoing prenatal diagnosis identified clinically significant structural variants with enhanced resolution and increased sensitivity to detect mosaicism compared with CMA testing.[119] Taken together, the increasing utilization of exome sequencing for prenatal testing and the continued technical improvements in genome sequencing suggest that in the future, prenatal genome sequencing may have clinical utility by coupling genome-wide CNV and structural variant detection with appropriate content and reporting restrictions. However, continued utility research and policy guidelines will be necessary before broadly adopting this approach to prenatal diagnosis, particularly concerning issues of equitable access, the necessary infrastructure for genetic counseling, policy development, fiscal sustainability, and associated ethical and psychosocial implications.

Genome sequencing and mitochondrial genetic testing

Mitochondria are directly involved in several important cellular processes, including oxidative phosphorylation (OXPHOS) energy production, apoptosis, cytosolic calcium level control, lipid homeostasis, steroid synthesis, innate immune response, and metabolic cell signaling. Importantly, all mitochondria harbor a circular 16.6 kb double-stranded DNA (mtDNA) that encodes 13 essential OXPHOS genes and the rRNAs and tRNAs necessary for their expression. The mtDNA is maternally transmitted and has a very high mutation rate, which can result in a spectrum of benign to pathogenic variants. Given that there are numerous mitochondria within a cell, mixed intracellular populations of wild-type and mutant mtDNAs can coexist (ie,

heteroplasmy), and their proportion can vary across tissues. Most pathogenic mtDNA variants are heteroplasmic and the manifestation and severity of disease is directly related to the heteroplasmy levels in affected tissues.[120] The thresholds for heteroplasmic disease vary across tissues; however, those tissues with high energy requirements are most commonly affected.

Mitochondrial disorders are genetically heterogeneous because they can be due to a defect in mtDNA or to pathogenic variants affecting OXPHOS complex subunits and/or assembly factors that are encoded by nuclear DNA (nDNA). As such, mitochondrial disorders can be sporadic, maternally inherited, or follow Mendelian inheritance.[121] The most comprehensive mtDNA disease sequence and variant resources include MITOMAP,[75] HmtDB,[122] HmtVar,[123] MtSNPscore,[124] MSeqDR,[125] and ClinVar, which provide curation resources for both mtDNA and mitochondrial nDNA variants. In addition, an international working group of the Mitochondrial Disease Sequence Data Resource Consortium recently adapted the ACMG/AMP constitutional variant interpretation guidelines[62] to provide guidance on mtDNA variant classification (Table 2).[60-76]

Traditional diagnostic methods for the detection of mtDNA variants include Sanger sequencing of prioritized genes and alternative methods to accurately quantify mtDNA heteroplasmy,[126] as well as large mtDNA deletion analysis by Southern blotting or custom CMA.[127] However, high-throughput sequencing of the complete mtDNA and selected nDNA genes has greatly improved mitochondrial diagnostics by enabling more accurate quantification of mtDNA heteroplasmy and better detection of deletions.[128] The clinical and genetic heterogeneity of mitochondrial disorders along with the growing number of implicated nuclear genes has led to exome sequencing as an effective first-tier platform for mitochondrial diagnostic testing[129,130]; however, most commercial exome capture kits do not contain probes that specifically interrogate the mitochondrial genome. Alternatively, "off-target" capture of the mitochondrial genome can be analyzed from exome sequencing data; however, the limitations of this approach include a greater depth of coverage needed for accurate quantification of low-level heteroplasmy and the inability to reliably identify mtDNA CNVs.[131] The diagnostic yield of exome sequencing for mitochondrial disorders ranges from 35% to 70% depending on the patient population.[132,133]

Genome sequencing provides further potential for mitochondrial diagnostics based on consistent coverage and simultaneous sequencing of mtDNA and nDNA, inclusion of noncoding regions, superior mtDNA coverage, and better detection of structural variants.[5,134] For example, a previous study on patients with suspected mitochondrial disease concluded that the diagnostic yield is at least equivalent to exome sequencing for known variants but with potential for improved yield because of identification of novel genes and variants.[135] More recently, genome sequencing was applied to a cohort of 345 patients with suspected mitochondrial disease, which resulted in a probable diagnosis in 31% of

cases, 38% of which were mitochondrial, and 63% were nuclear.[136]

Genome sequencing and pharmacogenomic testing

Pharmacogenomic testing is increasingly being considered for clinical implementation for selected medications with high levels of evidence (Figure 2[49]).[137] Important resources for pharmacogenomics include the Pharmacogenomics Knowledgebase (PharmGKB),[68] practice guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC),[138] and the US FDA Table of Pharmacogenetic Associations[139] (Table 2[60-76]). Importantly, the ACMG has recently reported a technical guideline on clinical pharmacogenomic testing and reporting, which includes recommendations when implementing exome- and genome-based pharmacogenomic testing,[140] and the AMP continues to release recommendations on which alleles to include in clinical pharmacogenomic genotyping assays for multiple genes with available CPIC/DPWG guidelines.[141-146]

Most clinical pharmacogenomic panels are based on targeted genotyping because of the cost-effectiveness of the platform, focused content selection, and relatively short turnaround time.[147] However, a limitation of targeted genotyping is detecting structural variants and CNVs unless they are directly interrogated by specific molecular assays,[148] which are increasingly identified as clinically significant forms of pharmacogenomic variation,[149,150] particularly for *CYP2D6*.[151-153] Pharmacogenomic full-gene sequencing[154] and enrichment-based exome and genome sequencing have also been reported[155]; however, these strategies are not able to phase haplotypes and diplotypes compared with the reported long-read pharmacogenomic sequencing assays.[156,157]

Enrichment-based exome or genome sequencing is not currently cost-effective for routine pharmacogenomic testing; however, these platforms have been analytically assessed across pharmacogenomic genes with CPIC guidelines.[155,158] Genome sequencing results in highly concordant variant calls compared with clinical pharmacogenomic genotyping; however, genes with known short-read sequencing alignment challenges (eg, *CYP2D6*, *G6PD*, and *HLA*) were the most common sources of discrepancy. Although assessment of *CYP2D6* copy number was also not robust by genome sequencing, recent computational tools have reported improvements in inferring *CYP2D6* CNVs and gene conversions from short-read sequencing data.[159,160] In addition, star (*) allele diplotyping tools are currently in development,[161,162] which enable the translation of sequence variant calls to common pharmacogenomic nomenclature based on haplotype definitions from the Pharmacogene Variation (PharmVar) consortium.[163] As such, interrogating pharmacogenomic variation by genome sequencing is currently feasible,[164,165] including the clinically actionable *HLA* region,[166] and it is expected that the necessary computational phasing and translation tools will continue to improve and enable clinical pharmacogenomic reporting from genome sequence data.

Genome sequencing and polygenic risk score testing

Fifteen years of genome-wide association study (GWAS) research has resulted in the discovery of numerous constitutional variants (common and rare) implicated in complex diseases and other human traits but with effect sizes that are individually small. However, the combinations of these predisposition variants for certain diseases are now emerging as potentially actionable polygenic risk scores (PRS), with the aim of providing useful information for disease risk stratification or prognosis.[167] PRS algorithms are currently available that leverage thousands or millions of variants and weighted sums of allele counts to generate a single number that is proportional to the risk for a given disease; however, their clinical validity and utility are still being established, with the most robust PRS algorithms emerging for cardiovascular disease and certain types of cancer.[168] In an effort to enable more standardized PRS research and facilitate translation into clinical care, the ClinGen Complex Disease Working Group and the Polygenic Score (PGS) Catalog recently reported Polygenic Risk Score Reporting Standards (PRS-RS) to inform PRS best practices and result reporting,[169] and the ACMG has recently reported a "points to consider" perspective on the development of laboratory-developed PRS tests.[170]

PRS testing is inherently a genomic assay, which was initially driven by GWAS that used high-resolution single-nucleotide variant microarrays; however, as high-throughput sequencing technologies became more accessible, GWAS programs evolved to utilize exome and genome sequencing, including more cost-effective low-pass (~1×) genome sequencing. As such, clinical genome sequencing is uniquely suited to support PRS testing because the consistent coverage at depths commonly implemented for clinical testing (>30×) can easily genotype single-nucleotide variants implemented in PRS algorithms. This conceptually suggests that, similar to optional pharmacogenomic reporting for diagnostic genome sequencing, PRS reporting for selected diseases could also be developed as a companion to Mendelian diagnostic testing when implementing a clinical genome platform. However, a more cost-effective strategy for independent PRS testing utilizes low-pass genome sequencing coupled with variant imputation, which has resulted in both efficient and accurate PRS prediction compared with both microarrays and high-depth genome sequencing.[171]

Despite the extensive research on PRS for many diseases and complex traits, clinical laboratory guidelines and best practices are only beginning to emerge. As such, development of clinical PRS tests (low-pass or high-depth sequencing) must follow the laboratory-developed test validation requirements from the CAP. The unique characteristics of PRS testing necessitate additional analytical considerations beyond variant calling accuracy and precision, particularly when pursuing low-pass genome sequencing. Several imputation methods are currently available to infer common variant genotype results from low-pass sequence data, and laboratories must assess which

is the most accurate and robust for their assay. Moreover, a quantitative assessment of PRS prediction using specimens with orthogonally derived PRS values that are considered truth is also necessary to validate all elements of the low-pass sequencing PRS workflow. However, although PRS testing is increasingly being considered by progressive laboratories that pursue clinical genome sequencing, caution should be exercised when implementing across diverse populations because most PRS algorithms have been developed among predominantly European cohorts.[172]

Clinical genome sequencing limitations

Technical limitations

Despite the analytical improvements with uniform coverage and small-variant/CNV accuracy of genome sequencing compared with enrichment-based exome sequencing,[173] genome sequencing does have some technical limitations. For example, short-read genome sequencing can have inconsistent CNV breakpoint identification, particularly for copy-number gains. Although the resolution of CNV detection is greater by genome sequencing compared with CMA testing, algorithms for CNV identification can result in imprecise breakpoint detection and false positives. This can be partially solved by filtering CNV calls using an internal database of genome sequencing-based CNVs; however, this effort inherently requires extra resources and a significant volume of internal data. Notably, long-read sequencing-based structural variant detection is rapidly emerging as a more accurate method for these constitutional events, particularly for complex structural variants.[55,174,175]

In addition, given the lower depths typically implemented with genome sequencing (~30×), mosaic variant detection by genome sequencing is less sensitive compared with higher depth enrichment-based panel or exome sequencing. Moreover, mosaic CNVs are also problematic for genome sequencing, particularly for mosaic aneuploidy (eg, mosaic Turner syndrome).[176] Another technical challenge for genome sequencing compared with enrichment-based sequencing is specimen types because interfering bacterial contamination in saliva samples is known to negatively affect certain genome sequencing quality metrics.[177] In addition, although genome sequencing has proven performance for mitochondrial disease testing, significant differences in detecting low-level heteroplasmic variants has been reported between genome sequencing and targeted mtDNA sequencing.[178]

Economic limitations

Importantly, the costs of sequencing are continually evolving and are dependent on multiple factors, including platform and chemistry, desired depth and sample pooling strategy, laboratory procedures and overhead, and intended use. As such, thorough comparisons between enrichment-based panels, exome, and genome sequencing were also considered outside of the scope of this review. However, as one of the most notable factors driving the implementation of genome

sequencing among clinical laboratories, direct reagent costs for short-read enrichment-based exome and genome sequencing (short and long read) at clinical-grade sequencing depths on high-throughput instruments can be currently estimated at ~$100 and ~$500, respectively. This does not include any computational, workflow, or technical effort considerations but does highlight an important barrier for clinical laboratories when implementing genome sequencing. However, higher throughput short- and long-read sequencing platforms have recently been released, indicating that individual genome sequencing reagent costs will only continue to decline.

Of note, the cost-effectiveness of genome sequencing for diagnosing critically ill infants and pediatric patients with suspected genetic disease has recently been reported to be potentially cost saving as a first-line diagnostic platform.[179-181] However, although preliminary economic evaluations support the cost-saving potential of diagnostic clinical genome sequencing, multidisciplinary implementation research, including more robust outcome measurement and economic evaluation, is needed to demonstrate the cost-effectiveness of clinical genome sequencing.[16,182] Moreover, although the reimbursement landscape for clinical genome sequencing has historically not been very favorable, this is also a rapidly evolving area that is now supported by a dedicated Current Procedural Terminology code for clinical genome sequencing (81425).

Conclusions and future directions

Genome sequencing is increasingly being considered as a single platform for genetic testing by clinical laboratories, which is based on the ongoing improvements in throughput, computational pipelines, variant calling and prioritization algorithms, structural variant callers, and the potential utility across many traditional constitutional genetic testing applications. Although professional guideline recommendations for genome sequencing are still emerging, substantial data currently exist supporting potential utility across diagnostic testing, carrier screening, molecular karyotyping, mitochondrial testing, and pharmacogenomic and polygenic risk score testing. This progressive approach is justifiably contrasted by important logistical and ethical considerations because the infrastructure and resources needed to implement clinical genome sequencing are significant. Development and validation of laboratory and bioinformatic procedures, as well as establishing a computational data management infrastructure, require investments that may not be feasible for many laboratories at this time; however, the efficiency of a single platform would undoubtedly result in significantly reduced long-term resources when developing "new" tests with a single genome sequencing workflow.

Although short-read sequencing bioinformatic tools are emerging to facilitate the accurate interrogation of historically challenging genomic regions, long-read genome sequencing inherently can outperform short-read sequencing across homologous regions, repeat expansions, and other difficult

sequence contexts. As such, future iterations of clinical genome sequencing may leverage the benefits of both technologies by integrating the throughput of short-read sequencing with the improved phasing and variant calling accuracy of long-read sequencing; however, additional research and validation data are needed to establish the clinical feasibility of this approach. New technologies are also increasingly being implemented by clinical genomic laboratories, including optical genome mapping, which is based on fluorescently labeled high molecular weight DNA molecules. Optical genome mapping has recently been reported to have improved structural variant calling and molecular karyotyping compared with classic cytogenetic technologies,[110,183] supporting its utility as an orthogonal cytogenomic platform. However, the rapid and ongoing improvements in both short- and long-read sequencing indicate that it is only a matter of time before genome sequencing as a single platform for clinical constitutional genetic testing is considered routine and not a progressive outlier.

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Conflict of Interest

A.S. is a paid employee of LetsGetChecked, Monrovia, CA, and holds equity in LetsGetChecked, Opus Genomics, and PathFinder Health. All other authors declare no conflicts of interest.

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