TRANSLATIONAL GENETICS

Molecular genetic testing and the future of clinical genomics

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Abstract | Genomic technologies are reaching the point of being able to detect genetic variation in patients at high accuracy and reduced cost, offering the promise of fundamentally altering medicine. Still, although scientists and policy advisers grapple with how to interpret and how to handle the onslaught and ambiguity of genome-wide data, established and well-validated molecular technologies continue to have an important role, especially in regions of the world that have more limited access to next-generation sequencing capabilities. Here we review the range of methods currently available in a clinical setting as well as emerging approaches in clinical molecular diagnostics. In parallel, we outline implementation challenges that will be necessary to address to ensure the future of genetic medicine.

Large-insert clone
A large haplotype fragment that is inserted into, for example, a bacterial artificial chromosome.

Oligonucleotide arrays
Hybridization of a nucleic acid sample to a very large set of oligonucleotide probes, which are attached to a solid support, to determine sequence, to detect variations or to carry out gene expression or mapping.

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Molecular diagnostic testing in a symptomatic individual has become increasingly sophisticated. Until recently, such testing was carried out on, at most, one or a few loci. The advent of large-insert clone arrays and, later, oligonucleotide arrays changed this landscape by allowing a patient's entire genome to be queried at improved resolution, thereby allowing the detection of medium to large genomic lesions. Today, this can be done at single-nucleotide resolution thanks to cheaper, faster and increasingly accurate whole-exome sequencing (WES) and whole-genome sequencing (WGS)[1]. Although genome sequencing is expected to transform diagnostics, non-sequencing molecular technologies remain crucial for efficiently and precisely screening and defining variation. Patients and families rely on molecular diagnoses for health-care management, disease prognosis and family planning, and they personally benefit when an answer is provided for the afflicting condition.

Amid the euphoria surrounding these advances, major analytical and interpretative challenges have emerged, ranging from the validation of large numbers of genomic changes in a patient, to the economic feasibility of this approach and its deployment in standard care, to managing the terabytes of data that accompany a single sequenced genome[2]. Deciphering the information that is locked in a patient's genome is not trivial. However, the effort invested towards development of informatic and molecular tools that are immediately applicable to both common and rare genetic disease has the potential to inform a broad range of clinical phenotypes[3–11].

Here we review a range of methods available for molecular diagnosis, their relative value for detecting genomic variation and some key challenges for each technology. With the rapidly changing technological platforms, we direct the reader to other articles for comparisons of next-generation sequencing (NGS) and other genomic technology reviews[12–14]. Further, we discuss the challenges of implementing these technologies into clinical practice, including policy development and ethical considerations. Although we concentrate our Review on laboratory testing in the United States, as it is a focal point for policy discussion and technological development, we present approaches to be considered in other countries and regions with more limited resources. We also focus on genetic testing for heritable genotypes or karyotypes as opposed to somatic mutations in cancer or viral load genetic testing. We do not cover newborn screening technologies, ancestry testing or identity DNA testing; for a scholarly discussion of prenatal genetic testing and ethical considerations, see REFS 15,16. We start with a discussion of the scope of genetic services and applications and current relevant technologies. We then focus on the challenging interpretation of genome variation, particularly in the nascent use of WGS and WES. Finally, we discuss the breadth of considerations and social implications of clinical genome sequencing, including access, ethics, genetics education and the regulatory landscape. At the conclusion of this Review, we discuss the upcoming challenges to integrating the next wave of genome sequencing into clinical practice.

Exome
The collection of protein-coding regions (exons) in the genome. As exons comprise only 1% of the genome and contain the most easily understood and functionally relevant information, sequencing of only the exome is an efficient method of identifying many variants that are likely to affect a trait.

Next-generation sequencing
(NGS). NGS platforms sequence as many as billions of DNA strands in parallel, yielding substantially more throughput than Sanger sequencing and minimizing the need for the fragment-cloning methods that are often used in Sanger sequencing of genomes.

The scope of clinical genetic testing

Genetic testing has grown from a niche speciality for rare disorders to a broad scope of applications for complex disease and personal use[17,18]. Not surprisingly, the definition of a genetic test has changed as the applications have evolved. Applications of clinical genetic testing span medical disciplines, including: newborn screening for highly penetrant disorders; diagnostic and carrier testing for inherited disorders; predictive and pre-symptomatic testing for adult-onset and complex disorders; and pharmacogenetic testing to guide individual drug dosage, selection and response (TABLE 1). Currently, genetic tests may be indicated in different clinical contexts and ordered by multiple health-care providers (see Further information for resources of available genetic tests). The circumstances of the individual genetic test — including the acute nature of the phenotype, the age of the patient, family history and specimen availability — guide the selection of tests and test platforms. For example, prenatal WGS can detect carrier status for a host of rare genetic disorders[19] but might be considered to be impractical for routine screening. Genetic tests in under-funded regions may continue to be driven by the candidate gene approach on the basis of the phenotype of a patient, as has been the paradigm in the United States for two decades. Still, these approaches hold a valuable role in certain classic monogenic syndromes and in families with a previously attributed molecular cause. However, in naive cases for genetic work-up, an argument could be made that (not accounting for cost) whole-genome analysis may be valuable in determining mutation load and identifying other genetic factors relevant to health planning.

Post-millennium genetic technologies

For the most part, clinical molecular diagnostic technologies remain focused on identifying patients' underlying pathogenic mechanisms. TABLE 2 summarizes the methodologies that are applicable to heritable genotypes and karyotypes. With direct genetic testing, the laboratory looks for the particular genetic variant (or variants) that contributes to a condition, whereas

Table 1 | **Factors considered in selecting a genetic test**

Test	Description	Example	Embryo or blastocyst (pre-implantation genetic diagnosis)	Fetus (prenatal testing)	Child	Adult
Newborn screening	Targeted tests for recessive genetic disorders	Phenylketonuria, cystic fibrosis, sickle-cell anaemia	Not applicable	Not applicable	Tests provided at birth vary by country and state or region	Not applicable
Diagnostic testing	Confirmatory test or differential diagnosis testing for a symptomatic individual	Skeletal dysplasias, thalassaemias, craniosynostoses	Specimen type and limited available amount for sampling may restrict platform selection (for example, WES or WGS versus SNP or STR typing)		Where treatment is desired, turnaround time may restrict platform selection	
			Turnaround time necessary may restrict platform selection			
Carrier testing	Targeted testing for asymptomatic individuals potentially carrying one or more recessive mutation	Cystic fibrosis, thalassaemias, Tay–Sachs disease	Applied typically for rare disease but applicable for other familial mutations		Carrier testing of minors is considered in the context of individual paediatric cases[164,165]	According to standard of care
Predictive testing	Tests for variants causing or associated with diseases or disorders with a hereditary component, usually with adult-onset symptoms	Most cancers, cardiovascular disease, diabetes	Some have discouraged genetic testing of asymptomatic minors for adult-onset conditions			According to standard of care
Pre-symptomatic testing	Tests for variants causing or associated with diseases or disorders known to be inherited in the family, often with adult-onset symptoms	Huntington's disease, haemochromatosis, Alzheimer's disease	Some have discouraged genetic testing of asymptomatic minors for adult-onset conditions[152,153]			According to standard of care
			Interpretation of VUSs will depend on presenting phenotypes in the family			
Pharmacogenetics	Targeted tests for variants associated with pharmaceutical dosage choice or adverse reactions	DNA tests for abacavir, warfarin, carbamazepine	Application not currently conducted but theoretically feasible	Application not currently conducted, but conceivably applicable for screening treatment approaches *in utero*	Pharmacogenetic testing is considered in context of individual paediatric cases[166]	According to standard of care

SNP, single-nucleotide polymorphism; STR, short tandem repeat; WES, whole-exome sequencing; WGS, whole-genome sequencing; VUS, variant of unknown significance.

Direct genetic testing
Testing that looks at the presence or absence of known genetic variants that contribute to pathogenicity.

Indirect genetic testing
Testing that compares the genetic regions of multiple affected persons to unaffected persons. Indirect genetic tests may evaluate patterns of inheritance in multiple family members with a known trait and look at the segregation of the trait with genetic markers.

Linkage analysis
A statistical method for identifying a region of the genome that is implicated in a trait by observing which region is inherited from the parental strain carrying the trait in offspring that carry the trait.

indirect genetic testing relies on the comparison of DNA markers that are linked to a trait of interest but that do not cause the genetic condition.

Every shift in technology is accompanied by the need to assess the quality and feasibility of the new platform for diagnosis. (TABLE 3 defines the terms that are useful for evaluating diagnostic tests.) Analytical validity is a measure of the ability of a molecular test to detect a genetic or genomic variant, both in terms of the analytical sensitivity of the assay (false-negative rate) and the analytical specificity of an assay (false-positive rate). By contrast, the clinical validity refers to the ability of the test to predict the presence or absence of a clinical condition.

Indirect testing. Despite the surge of new technologies to interrogate disease-causing variants in a patient in well-funded laboratories, indirect methodologies continue to have a prominent role in diagnostics in regions of the world with more limited resources (and thus a substantial fraction of the human population); in particular, linkage analysis using single-nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) can be applied[20]. Classical indirect approaches (for example, single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and heteroduplex analysis) have mostly been phased out in the United States, but these techniques are still highly used in developing regions with limited resources[21–23]. In some cases, indirect tests could inform whole-genome data (see discussion below) by narrowing in on regions of interest; this is an approach that is commonly used in research to save costs of WGS[20]. Further, for some specialized applications, such as non-invasive prenatal testing (NIPT) and pre-implantation genetic diagnosis (PGD), the ability to amplify and to differentiate STRs from trace samples or even single cells makes microsatellite linkage analysis an attractive approach[24–26].

Targeted allele-specific mutation detection. Amplification combined with restriction digest, hybridization or another means of detecting a mutation remains among the cheapest and most robust methods in clinical molecular diagnostics. The simplicity of PCR mutation detection makes throughput of multiple samples feasible and offers high confidence to detect variants. For example, common disease-causing repeat

Table 2 | **Clinical genetic testing methodologies**

Method	Common point mutations	Rare point mutations	Copy number variants	Uniparental disomy*	Balanced inversions or translocations	Repeat expansions	Analytical sensitivity‡§	Analytical specificity‡‖	Turnaround time‡¶	Cost#	Examples
Linkage analysis (commonly STRs)	X		X**				Low	Low	Low	Low	Historical familial mutation
FISH			X		X		Low	Low	Low	Low	Angelman's syndrome
Array CGH or virtual karyotyping			X	X			Average	Average	Average	Average	A new referral or challenging diagnostic case
Genome-wide SNP microarrays	X		X				Low	Low	Low	Low	Cardiovascular disease risk assessment
Target PCR	X	X**				X	High	High	Low	Low	Cystic fibrosis carrier testing
Sanger gene sequencing	X	X					High	High	Average–high	Average	Treacher Collins syndrome diagnosis
Southern blot or MLPA			X			X	High	High	High	Low	Fragile X syndrome
Panel or pathway sequencing	X	X					Average	Low	Average	Average	Long QT syndrome
WES or WGS	X	X	X‡‡				Low	Low	High	High	A new referral or challenging case to diagnose

CGH, comparative genomic hybridization; FISH, fluorescent *in situ* hybridization; MLPA, multiplex ligation-dependent probe amplification; SNP, single-nucleotide polymorphism; STR, short tandem repeat; WES, whole-exome sequencing; WGS, whole-genome sequencing. *Familial mutations or genomic rearrangements can be assayed. ‡Categorical assignments in these columns are subjective and vary according to context of the tests being ordered and the laboratory conducting the tests. The 'low', 'average' and 'high' are presented to simplify and to compare platforms generally. §Low, <80%; average, 80–98%; high, >98%. ‖Low, <80%; average, 80–98%; high, >98%. ¶Low, <1 week; average, 1 week–1 month; high, >1 month. #Costs of the testing will widely vary from one laboratory to the next; however, these estimates are based on the charge of the test from a sampling of laboratories, not on the costs of consumables or the reimbursed amount. Low, less than US$400; average, $400–$2,000; high, >$2,000. **Uniparental disomy can be detected by any method if both parents are genotyped. However, only the indicated approaches will detect uniparental disomy in absence of the parental genetic samples. ‡‡Copy number variant detections are improving in next-generation sequencing applications but are more efficient in WGS than WES, although they are of limited reliability for clinical diagnostics.

Table 3 | **Evaluating the validity of genetic tests**

Term	Definition	Complications in molecular tests	Calculation
Analytical sensitivity	Refers to the proportion of assays with the genotype that have a positive test result (false-negative rate of the assay)	Allele drop out; preferential amplification; mosaicism	True positives / (true positives + false negatives)
Analytical specificity	Refers to the proportion of assays without the genotype that have a negative test result (false-positive rate of the assay)		True negatives / (true negatives + false positives)
Clinical sensitivity	Refers to the proportion of people with a disease who have a positive test result (false-negative rate of diagnosis)	Variable penetrance; variable expressivity	True positives / (true positives + false negatives)
Clinical specificity	Refers to the proportion of people without a disease who have a negative test result (false-positive rate of diagnosis)		True negatives / (true negatives + false positives)
Positive predictive value (PPV)	Refers to the likelihood that a patient has the disease given that the test result is positive		True positives / (true positives + false positives)
Negative predictive value (NPV)	Refers to the likelihood that a patient does not have the disease given that the test result is negative		True negatives / (true negatives + false negatives)
Clinical utility	Refers to the value of the test for determining treatment, patient management and family planning	Depends on health-care system and environment	Subjectively determined on the basis of reports supporting use and economic benefits
Personal utility	Refers to the value of the test for personal and family choices	Depends on personal vantage	Subjectively determined from an individual's perspective

expansions, such as those in fragile X syndrome, are frequently tested for by direct amplification of the repeated fragment[27]. This approach is ideal for carrying out simple assays on common variants, such as a Taqman® assay for genotyping a pharmacogenetic variant or factor V Leiden mutation. The disadvantage of allele-specific PCR is, of course, the inability to detect any relevant variants that have not been assayed. Nonetheless, these approaches retain a high value, especially in laboratories with limited resources and/or access to advanced instrumentation and are likely to remain core clinical assays.

Gene-specific Sanger sequencing. For detection of point mutations and small variants, bidirectional Sanger sequencing has been considered the 'gold standard' in clinical genetic testing for the past decade[28]. This direct approach has high analytical validity (TABLE 3), although long reads can deteriorate quality for base calling, and minute specimens can produce PCR artefacts[29,30]. The fundamental value in directly sequencing one or more entire genes is the ability to combine a clinical indication for a candidate gene with the high sensitivity and specificity of the assay (TABLE 3). For instance, focused sequencing of a single gene (namely, *FGFR2*) can confirm or rule out a diagnosis of Apert's syndrome at fairly low cost[31], sequencing *TCOF1* will to detect up to 90% of mutations in patients with Treacher Collins syndrome[32], whereas testing six genes known to cause Noonan's syndrome (namely, *PTPN11*, *SOS1*, *RAF1*, *NRAS*, *CBL* and *KRAS*) detected mutations in 30% of individuals with clinical features suggestive of Noonan's syndrome[33]. As the analytical validity of whole-genome technologies improves (TABLE 3), genome sequencing will probably become the first-pass instrument of genetic analysis to inform candidate gene Sanger sequencing (see below). It is important to note that although Sanger sequencing is of high analytical validity, the clinical validity of the approach is dependent on the genetic drivers of a condition. Sanger sequencing does not detect most structural changes, so it alone is not sufficient for diagnosis for many genetic disorders.

Genome-wide SNP microarrays. Microarray-based genotyping can be divided into three main applications: array comparative genomic hybridization (array CGH) to detect structural anomalies (see discussion below), phenotype-specific SNP panels, and genome-wide SNP panels. Efforts in academic and commercial laboratories have produced phenotype-specific panels containing alleles that are known to drive specific phenotypes, such as panels for retinal degeneration[34,35]. The utility of this approach is that a low-cost, expeditious experiment interrogating multiple genes can offer high-quality molecular diagnoses. However, the continuous discovery of novel causal alleles and genes, as well as variable penetrance and expressivity of known mutations[36] limits the clinical validity of this approach (TABLE 3).

By contrast, large-scale genome-wide SNP genotyping offers a single, cost-efficient platform to assess risk of multiple common genetic disorders with variably documented associations in one test[36–38]. Predictive and presymptomatic testing is available as a multiplex platform for a host of conditions, including certain cancers and pharmacogenetic tests, as well as for ophthalmologic, cardiac, renal and neurological disorders (among others). Several personal genome companies now provide versions of commercial clinical genotyping services to consumers, such as the Personal Genome Service from 23andMe, Pathway Genomics and Navigenics, to name but a few[39]. Although the tests are designed for and available to consumers, because the analytical tests are conducted in clinical (that is, CLIA-certified) laboratories, such genome-wide SNP tests may also be ordered by clinicians. With genome-wide SNP tests, particular loci

Single-nucleotide polymorphisms
(SNPs). Differences in the nucleotide composition at single positions in the DNA sequence.

Short tandem repeats
(STRs). DNA sequences containing a variable number of highly polymorphic, tandemly repeated short (2–6 bp) sequences.

Non-invasive prenatal testing
(NIPT). A method of obtaining a prenatal diagnosis by detecting fetal cells circulating in maternal blood.

Pre-implantation genetic diagnosis
(PGD). An *in vitro* method of identifying genetic defects in *in vitro* fertilization embryos before maternal transfer and implant.

Sanger sequencing
A method used to determine the nucleotides present in a fragment of DNA. It is based on the chain terminator method developed by Frederick Sanger but currently uses labelling of the chain terminator dideoxynucleotides, allowing sequencing in a single reaction.

may be evaluated with high analytical validity (TABLE 3), but the limited scope of variant detection confines analysis to pre-selected points in the genome. Further, most SNP-based diagnostics are probabilistic, not deterministic, with variable degrees of clinical validity[40], as arrays identify a limited range of variants. For instance, homozygosity of common alleles at the two major loci for age-related macular degeneration (AMD; namely, *CFH* and *HTRA1*) have a high probabilistic value for disease onset[41–45] and might induce behavioural modification in patient management owing to the documented high association of the homozygosity of some SNPs and smoking[46], but the test has limited ability to predict AMD per se. Newer hybrid platforms, such as exome chips that contain all known coding variants reported both in patients and in control individuals might offer improved efficiency in identifying the mutational load of patients for both rare and common alleles that are relevant to disease status, although they too might have limited clinical validity[39].

Detection of structural and chromosomal variation. Recent improvements in chemistry and microscopy have substantially augmented the resolution of cytogenetics, most notably through the development of multi-probe fluorescent *in situ* hybridization (FISH; for a detailed review, see REF. 47) and chromosomal CGH. Economic factors aside, cytogenetic methods are gradually being phased out in the clinic in favour of a combined SNP–array CGH approach that uses probes to detect chromosomal and genomic rearrangements as well as deletions with greater precision and smaller genomic variations than FISH (for a thorough review of structural variation and medical genomics, see REF. 48). Depending on design and probe density, array CGH can offer resolution from whole chromosomes to deletions and duplications of a few kilobases in size[49]. Array CGH imparts improved sensitivity (TABLE 2) of rearrangement detection (with the important exception of balanced inversions and translocations) and the ability to detect readily uniparental disomy (UPD), which is not detectable through chromosomal CGH. At the same time, improved resolution has been accompanied by a massive increase in detection of submicroscopic genomic rearrangements of unclear importance to the clinical phenotype of tested patients. Resources such as Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) and International Standards for Cytogenomic Arrays Consortium (ISCA Consortium) are cataloguing submicroscopic deletions and duplications that may affect the copy number of dosage-sensitive genes or disrupt normal gene expression, leading to disease. These databases provide a common depot for aiding interpretation of the often novel and often *de novo* structural changes found in diagnostics[50,51]. Even so, non-uniform deposition of phenotypic data deposited represents a substantial limitation to the utility of such databases.

In parallel, other molecular techniques have exponentially improved in their ability to detect subchromosomal rearrangements of varying sizes and complexity. Southern blotting, which used to be widely used in combination with restriction fragment length polymorphism (RFLP) for molecular diagnoses, continues to be used to detect small genetic changes as well as large repeat variants that are not amenable to PCR amplification (for example, *FMR1* expansions)[27]. However, more recently, multiplex ligation-dependent probe amplification (MLPA) assays have replaced Southern blotting for some applications. In addition to standard copy number variants (CNVs), MLPA can detect mosaic mutations, as well as methylation status[52]. Further, MLPA can be used to confirm structural anomalies detected by FISH or CGH[53]. However, in most cases MLPA does not detect balanced genomic rearrangements, such as translocations or inversions[52], which is a substantial limitation, given the emergent realization of these types of events in human genetic disease[54]. We anticipate that for some types of genetic lesions, such as large trinucleotide expansions, classical molecular methods, including Southern blotting and MLPA, will remain assays of choice.

Whole-genome and whole-exome sequencing. NGS uses powerful massively parallel sequencing assays to sequence many genes of interest, the whole exome or the whole genome for variants in a broad range of rare and complex disorders. Targeted exon capture before genome sequencing (that is, WES) facilitates efficient analysis of most of the coding regions of the genome, whereas WGS evaluates almost all of the euchromatic human genome (it is important to note that heterochromatic regions will remain off limits for some time until read lengths become long enough to resolve repeat-dense regions). WES has proven to be a fast and accurate discovery approach for some mutations causing Mendelian disorders[3,5–10,55–59]. The plummeting cost of genome sequencing is reducing reagent costs below those of Sanger sequencing for some candidate genes (this is especially true for focused gene panels), making application of WES and WGS economically feasible[60,61]. At this time, WGS is not clinically available, but WES is available from select clinical laboratories (for a scholarly discussion of WES and WGS in clinical diagnostics, see REF. 62). Interpretation of clinical WES is limited, with few reported results clinically actionable. However, medical geneticists at major academic centres now routinely counsel for and order WES for unexplained genetic disorders. Although the choice between the two technologies is primarily driven by cost, after WGS has been offered as a clinical service, WGS is expected to supersede WES in the coming years, at least in well-funded arenas. Naturally, as with every disruptive technology, WGS data will introduce a new challenge over WES of interpreting non-coding variants that may contribute to the genetic load of a patient's phenotype.

In some cases, a targeted NGS approach based on a suspected syndrome may be taken to minimize costs and to maximize variant identification (for a review of disease-targeted sequencing, see REF. 63).

In addition to its use in WES and WGS for diagnostics and discovery, NGS can be used to detect methylation status, alternative splicing, small RNAs, allele-specific expression and even haplotypes and rearrangements[64–67].

Array comparative genomic hybridization
(Array CGH). A microarray-based method of identifying differences in DNA copy number by comparing a sampled genome to a reference genome.

Penetrance
The proportion of individuals with a given genotype who display a particular phenotype.

Fluorescent *in situ* hybridization
(FISH). A molecular and cytogenetic method using a fluorescently labelled DNA probe to detect a particular chromosome or gene using fluorescence microscopy.

Uniparental disomy
(UPD). An occurrence of an individual inheriting both copies of her chromosome from one parent.

Restriction fragment length polymorphisms
(RFLP). Variations between individuals in the lengths of DNA regions that are cut by a particular endonuclease.

Multiplex ligation-dependent probe amplification
(MLPA). A molecular technique involving the ligation of two adjacent annealing oligonucleotides followed by quantitative PCR amplification of the ligated products, allowing the characterization of chromosomal aberrations in copy number or sequence and single-nucleotide polymorphism or mutation detection.

Copy number variants
(CNVs). Structural genomic variants that result in copy number changes in specific chromosomal regions. Usually, there are two copies of each locus, but if, for example, duplications or triplications occur, then the number of copies will increase.

Although it has not yet been fully vetted for applications other than WES and WGS, NGS could potentially become a robust platform for a range of other 'omics' applications.

Cost considerations notwithstanding, the primary practical barrier to the use of WES and WGS in clinical settings is the limited ability of the technology to detect reliably the absence or presence of mutations. Different sequencing platforms have been shown to deliver results of variable quality, with some instruments more accurate at individual base calls and others covering a broader range of the genome[68–70]. Targeted approaches, including specific gene panels and whole exomes may be of greater analytical sensitivity (that is, they have a better coverage of the target to detect heterozygous changes) but restrict the clinical sensitivity (TABLE 3) in comparison to WGS, which might limit the interpretive scope to coding lesions. Even then, it is not possible at present to obtain high-quality sequence from the entire human genome, or even the euchromatic genome, that is sufficient for exhaustive clinical interpretation.

Further, to parse sequencing data efficiently, WES and WGS efforts for diagnosis often include sequencing of the proband and both unaffected parents in a trio to ascertain efficiently *de novo* and inherited mutations under limited information with regard to the mode of inheritance. With interpretation limited in current WES clinical tests to *de novo* and previously reported variants, some clinical WES laboratories sequence only the proband and confirm variants of interest in the parents. Nonetheless, access to the biological relatives remains valuable in interpretation of genetic variation. To refine further the vast amounts of data, confirmation testing by Sanger sequencing in probands and family members is typical. We are optimistic that the technical challenges of WES and WGS will be solved as market forces and clinical needs drive the field forwards. However, there remain acute interpretive problems that are dependent on the scope of the initial genome analysis (see the discussion of interpretive challenges below).

Taken together, the economical and analytical constraints of these technologies will limit WES and WGS to being an attractive first step in differential diagnosis, requiring secondary confirmations and possibly parallel testing by other methods for some time to come[71].

Evolving results

The success cases in rare diseases of WES are promising[72–74]; however, routine clinical genomic sequencing is waft with complications, resulting from both its unprecedented scale and interpretive challenges. Reliable interpretation of the multiple and *de novo* variants found through NGS will require additional experience and validation before it reaches the clinic on a large scale, particularly for diagnosis of complex traits[75,76]. Nonetheless, the clinical implementation of WES and WGS will probably transform clinical genetic testing, especially after genome-wide data become integrated into electronic medical records (EMRs)[77–79]. After this transition has occurred, specialized, phenotype-driven tests will probably wane and eventually disappear, and molecular diagnostics will focus instead on the interpretation of existing data[80].

Until recently, genetic data did not drive diagnosis but had a primarily confirmatory role. Moreover, the knowledge of pathogenic lesions typically leads to population-based arguments about possible patient outcomes. A major challenge is to convert pathogenic genetic data into a primary diagnostic tool that, in combination with clinical observation and biometric data, can shape clinical decisions and long-term management in a proactive way. Most emerging clinical genome-sequencing paradigms focus on a narrow phenotypic band in order to probe its genetic architecture in detail. A broader approach — namely, sequencing and parsing the total load of variants irrelevant to phenotype — will contribute meaningfully to what is a core question: should we sequence every patient admitted to a hospital and, if so, how do we interpret these data for clinical use?

Causal disease variants. In clinical diagnostic genetic testing, the American College of Medical Geneticists[81] recommends that variants be assigned to one of the following six categories:

- 'Disease causing': sequence variation has previously been reported and is a recognized cause of the disorder (for example, deletion of F508 in *CFTR*);
- 'Likely disease causing': sequence variation has not previously been reported and is of a type expected to cause the disorder, usually in a known disease gene (for example, a nonsense mutation in a gene for which other mutations of this type, but at a different residue, have been reported);
- 'Possibly disease causing': sequence variation has not previously been reported and is of the type that may or may not be causative of the disorder;
- 'Likely not disease causing': sequence variation has not previously been reported and is probably not causative of disease;
- 'Not disease causing': sequence variation has previously been reported and is a recognized neutral variant;
- 'Variant of unknown clinical significance': sequence variation is not known or expected to be causative of disease but is found to be associated with a clinical presentation.

Most of these categories of variants are subject to additional interpretation on the basis of literature, population frequencies, clinical findings, mutation databases and possibly case-specific research data. In addition, a variant may be considered to be protective or related to drug response.

Interpreting sparsely documented genetic mutations lacking evidence in co-morbidity has been challenging since the outset of molecular diagnostics, relying historically on the segregation patterns of inheritance, statistical incidence of a variant and the conservation of the altered amino acid in non-human species. To complicate this problem further, some laboratories responsible for assigning the importance of a molecular finding make decisions largely on the basis of experience of that laboratory in the analyte of interest. The current onslaught of

Box 1 | **Variant interpretation: a case study**

Consider an individual with a family history of amyotrophic lateral sclerosis (ALS), which is a lethal disorder with no treatment options (the index has two brothers, both of whom died at the age of 50). Motivation for the test is both for family planning but also for personal life planning. Whole-exome sequencing (WES) identifies a known mutation in superoxide dismutase 1, soluble (*SOD1*), one of the known ALS genes for which the confidence level for its pathogenic potential is high, given that the allele has been seen in other patients and confirmed in the deceased brothers from the index case. However, the test also detected a heterozygous nonsense mutation in ciliary neurotrophic factor (*CNTF*) that was deemed insufficient to drive disease as neither deceased brother carried it, and it was found in three control exomes. Under most simplistic models in effect today, alleles with such characteristics might not be reported to the index, as they are variants of unknown significance (VUSs) and might be interpreted as not being 'medically relevant'. However, studies in model organisms and humans have shown that haploinsufficiency at *CNTF* can have a potent effect on the age of onset of ALS, potentially reducing a patient's lifespan by two decades[145].

WES and WGS data requires a sophisticated and transparent exchange of variants associated with detailed phenotypes or clinical indications. Disease-centric mutation databases have morphed into human disease variant databases that are valuable for documenting clinical variation, such as the Human Gene Mutation Database (HGMD)[82] and the hand-curated databases ClinVar and MutaDatabase[83]. With concentrated effort, these latter pilots could expand into the broader, focused exchange necessary to facilitate interpretation of both rare and common variation across varying platforms and laboratories around the world.

Variants of unknown significance. We now recognize that with hundreds of loss-of-function variants and thousands of variants of unknown significance (VUSs) in each person's genome[84,85], prioritizing variants remains a primary challenge[56]. Genetic filters are of modest value, and with a shortened list of variants of interest, it is possible to enrich for specific variants[86], to analyse multiple family members, to examine concordance in computational algorithms[87–89] and to parse the morbid human and mouse genomes for variation[82,83]. Additional uncommon alleles may also be compared to human disease gene[90,91] and model organism databases[92,93]. These narrow approaches fail to take into consideration the potentially clinically useful trove of data from a WES or WGS experiment and are subject to a high false-negative rate for various reasons, including poor quality of sequence within a particular gene, mutational mechanisms not easily detectable by this technology and technical biases inherent to each instrument used[94].

Even in the context of a single gene or rare disorder, variant interpretation remains problematic, as a substantial fraction of alleles have poor predictive value, whereas modifier alleles are often excluded from consideration even though they can have profound phenotypic effects (BOX 1). This issue is amplified in genome-wide data. At present, for alleles that had not previously been associated with human pathology or for which there is limited biological insight (for example, model organisms and biochemical studies), *in silico* prediction algorithms (such as PolyPhen, VAAST and ESEfinder)[87,95–97] represent a common source of interpretation, and such analyses can be incorporated into clinical reports. This is problematic for two principal reasons. First, more commonly than not, such interpretations are taken at face value without an appreciation of the caveats and limitations of each algorithm. Second, the community has no metrics on the specificity or sensitivity (TABLE 3) of each of these programs to guide us with regard to possible false-positive and false-negative interpretations.

One solution that is currently in place exclusively in the research setting is the deployment of physiologically relevant functional assays that, in essence, 'functionalize' the morbid human genome. Such tools already exist for a small subset of disorders, most notably metabolic disorders, disorders of mitochondrial function and a handful of other conditions[98,99]. In addition, research studies ranging from protein stability studies to transcriptional activity and allele- and/or gene-specific animal models (for example, mice, fish, worms and flies) have all been used on multiple occasions to investigate the pathogenic potential of alleles relevant to clinical mutation findings[99–104]. However, no clinical laboratories can or do carry out such tests, and the challenge remains for functional annotation to be incorporated into clinical-grade interpretation of results. We do not envisage a time when such non-human studies will become bona fide clinical tests, as not only will they remain expensive, labour-intensive, difficult to automate and challenging to interpret in the context of human mutation, but they are outside the scope of existing regulatory guidelines in the United States (see the section below and BOX 2 on the regulation of genetic tests). Our hope is that clinical testing laboratories may collaborate with functional modelling laboratories to inform the variant findings. Consensus guidelines might be developed to annotate the hundreds of unique and/or rare alleles identified in patient genomes — and their functional consequences — in a fashion that will allow improved interpretation of genome variants and the introduction of such annotations into EMRs for patient use and health management.

Other considerations

Ethical considerations. The application of WES and WGS in the clinic has appropriately generated substantial debate in the community with regard to the delivery and impact of the information on physicians, patients and society in general[105–107]. Much consideration has been given to the ethical implications of genomic information provided to research participants (for example, see REFS 108–110), but less is known about the implications in a clinical setting[111,112]. BOX 2 discusses two of the key issues: how to handle secondary findings in whole-genome data and genetic privacy concerns.

Genetic education. Keeping pace with emerging clinical genetic technologies requires specialized genetic training as well as broad genetic literacy for patients and clinicians ordering and receiving genetics test results. In reality, genetics literacy in the United States is sorely lacking from elementary school through to medical

Variants of unknown significance (VUSs). Alterations in the sequence of a gene, the significance of which are unclear.

Box 2 | **Ethical considerations for genetic testing**

Secondary findings in genomic data

For most patients, whole-exome sequencing (WES) and whole-genome sequencing (WGS) will identify one or more novel (or rare) variants that are suspected to be disease-causing mutations but may also identify mutations that are relevant to adult medical care (for example, breast cancer and Alzheimer's disease)[146,147]. Although there is no consensus on whether and how to share this information with a patient, there is broad agreement that results must be confirmed by a clinical laboratory before returning to a patient[81]. Some advocate returning only results with certain findings of high medical importance, whereas others have proposed tiered return of results on the basis of relative risk[55]. Some clinical laboratories are exploring informed consent models to allow patients to elect what information to disclose. The Personal Genome Project, although not a clinical test, has taken the approach to return all secondary findings requested by the participant and to make WGS data on all participants publicly accessible[148,149]. Ultimately, the duty to inform patients of predictable risks could be influenced by the legal pressure and threat of malpractice[150].

Paediatric genetic testing raises the additional ethical challenge of deciding whether to test or to disclose results for adult-onset genetic conditions. There is no consensus on whether to withhold genomic information on a minor until he or she is of a consenting age to receive the data personally[151]; some policies discourage genetic testing of asymptomatic minors for adult-onset conditions such as Huntington's disease[152,153]. Longitudinal studies chronicle adverse events on minors receiving genetic test results[153], and predictive testing for Huntington's disease demonstrates minimal harm and a need to individualize to a patient's needs rather than to develop blanket policies[151–156].

Privacy and discrimination

A network of country-specific legislation protects Europeans from life and health insurance discrimination on the basis of genetics[157]. In the United States, clinical test results are subject to Health Insurance Portability and Accountability Act (HIPAA) protections; however, the HIPAA rule does not explicitly provide privacy protections for genetic information[158]. The US Genetic Information Nondiscrimination Act (GINA) of 2008 addresses this oversight to some extent[159]. GINA prohibits genetic discrimination in most health insurance and employment scenarios. However, the provisions do not apply to life insurance, disability insurance or long-term care insurance[158]. Despite the HIPAA and GINA protections, the public remains nervous about genetic information being used against them[160], and physicians are wary of genetic information being included in medical records[161]. As the applications and utility (both clinical and personal) of genetic testing expand, so too does the risk that discovered genetic information could be used against individuals. The protections of the existing US legal framework assuredly will be tested in courts. In the meantime, one key issue is how and where the delicate data resulting from WES and WGS clinical tests are hosted.

training[113,114,115]. By and large, the US public views genetics through the lens of genetic determinism. For the public to gain an understanding of polygenic inheritance and complex traits, primary and secondary genetics education must move beyond the mathematics of 'one gene, one phenotype' Mendelian inheritance[116] and embrace concepts of complex inheritance. Implementation of genomic sciences into clinical applications requires that clinicians be sufficiently versed in genetics and genomics to prevent that the result of these tests are misunderstood or misused[113,117]. The distinct role of the genetic counsellor in the genetics profession is extremely valuable in translating genetics and genomics concepts. However, the dearth of professionals trained for this role necessitates centralized telemedicine to provide broad access to genetics services[118]. Recent efforts to push genetics curricula into medical and nursing schools to attract professionals led to the successful development of core genetics competencies in nursing and medicine[113,117,119].

Genetic determinism
The idea that genes and genetic variants are the primary factor determining and shaping human traits.

Epigenomics
Describes a heritable effect on chromosome or gene function that is not accompanied by a change in DNA sequence but rather by modifications of chromatin or DNA.

Regulatory policy and standards. Regulating genetic tests continues to challenge authorities attempting to protect patients and consumers from misguided misuse of genomic technologies. This is not a new issue but one that continues to complicate existing models for regulating analytical and diagnostic tests in the United States (BOX 3) and around the world. With the emerging availability of WES and WGS in the clinic, the challenges are multiplied in some regards. For one, the analyte-specific model for regulating tests is no longer practicable when thousands or billions of analytes are assayed in a single clinical test. In addition, the burden on the regulator is evolving into one for regulating interpretation of rather than execution of results, authority for which is not clearly defined for genetic tests.

Mode of delivery. Until recently, physician and patient information exchange has been asymmetrical, if not paternalistic: patients are expected to adhere to regimens prescribed by a physician. However, it is clear that people with Internet access will seek medical information online[120,121], refuting the idea that patients want only a small amount of information or nothing more than a prescriptive regimen. We also know that the rise of 'crowd-sourced' patient websites (see Further information for resources) fulfils a need that is not otherwise being met by the traditional health-care system[122–126]. We expect crowd-sourcing to raise funds for rare disease testing or to create online communities to be integral to genetic interpretation on a personal level. Current evidence indicates that most people want to know their genetic test results and want choice in whether and how to access this information[109,127–131]. With increasing public interest in and attention to genetic services and decreasing availability of genetic experts to filter the information, patients are likely to seek their own modes of information gathering.

As genome sequencing enters the clinical realm, we must develop ways to communicate relevant findings to best inform clinical practice while remaining alert to the dangers of genetic determinism. Genetic variants that appear to precipitate a phenotype may also depend on environmental factors, modifier genes, epigenomics and the additive and synergistic effects from multiple variants[57]. Even simple genetic test results can be misunderstood in clinical translation[132]. Thus, communicating complex genomic results with a range of interpretations is challenging to say the least.

Costs, coverage and implementation. The availability of clinical genetic diagnostics in the United States depends on the practicability of both development of laboratory tests and payment for laboratory services. Clinical diagnostic laboratory directors select tests for development that will fit into existing throughput platforms, maximize efficiency and costs, and be subject to minimal competition. Laboratories that hold gene patents or that have exclusive licences for genetic testing benefit from such intellectual property by restricting test development and offerings by competing laboratories[133,134]. Newer technologies carry the additional costs of validation of novel platforms for clinical use, whereas WES and WGS in particular carry

Box 3 | **US regulatory policy and standards for genetic testing: a case study**

The vast majority of genetic tests offered in the United States are laboratory-developed tests (LDTs; sometimes called 'home brew' tests). In the United States, the Center for Medicare/Medicaid Services (CMS) currently regulates the analytical validity of LDTs through oversight of clinical laboratories under the Clinical Laboratory Improvement Amendments (CLIA) of 1988. CLIA certification is determined and maintained through CMS or through an independent accrediting body to verify quality standards and proficiency testing (for example, the College of American Pathologists and The Joint Commission). Genetic testing is not a speciality under CLIA so is usually regulated as a high-complexity chemistry test[162]. The US Food and Drug Administration (FDA) holds discretionary power to enforce oversight of LDTs and reviews *in vitro* diagnostic (IVD) devices (or assays) marketed commercially. Several states provide additional state-specific oversight of LDTs, and New York State requires evaluation of clinical validity for state certification. Recent focus on regulation of genetic tests stem in part from the advent of direct-to-consumer marketing and offering of personal genetic and genomic tests[163]. False claims of validity or utility of genetic tests are subject to Federal Trade Commission (FTC) enforcement. In addition to regulatory authority, guidelines for testing may be developed by professional organizations, such as the American College of Medical Genetics, for both rare disease diagnostics and broader technological platforms designed for risk prediction.

The analytical validity of most genetic tests is fairly high in comparison to other chemical assays subject to CLIA certification. However, the clinical validity can vastly vary depending on the genotype and the corresponding phenotype. As such, the crux of regulation of genetic tests lies not with the evaluation of the analytical validity of the IVD device or laboratory-developed test (LDT) but with the interpretation of any discovered genomic variants in context of a particular patient and a particular phenotype. However, clinical validity is not evaluated under CLIA and only claims of an IVD device are reviewed by the FDA. Moreover, newer NGS technologies (for example, microarrays and whole-exome and whole-genome sequencing) interrogate tens of thousands of analytes rather than a single or a few analytes. This substantially complicates the review processes of laboratory tests conducted both by the FDA and CMS. It is unclear at this point how to develop sufficient evidence for test validation, what controls are appropriate for such tests and how to establish proficiency routinely within a laboratory.

substantial costs in long-term data storage and informatics for interpretation of genomic variation. Reimbursement of genetic testing services by payers depends on the level of evidence for clinical utility (or it should do), the impact of such services on clinical decision making and the cost-effectiveness of genetic testing for a diagnosis[135–138]. With these economic constraints, diagnostic tests for rare diseases are not as commercially profitable as the tests for common disorders, given the expense of validation and proficiency testing. Integration of clinical diagnostics into practice depends on the speciality that is being considered for testing, but clinical decision support tools are vital for introducing testing options into hospital and outpatient workflow, particularly within EMRs[139,140].

Conclusions

The continued erosion of sequencing costs, driven in part by increased capacity of existing technologies and improvements in chemistry, as well as the emergence of single-molecule third- and fourth-generation sequencing[141,142], such as nanopore sequencing[64], suggest that in the fullness of time, most patients entering the healthcare system will have had their genome sequenced before clinical evaluation. Therefore, the composition of genetic testing will be fundamentally altered to focus on interpretation of genomic data in the context of an individual, their immediate and long-term needs, their personal choices and their environment. This will not be an overnight revolution, not least because it will be some time before emergent genomic technologies are of a sufficient quality and of a low enough cost to be accessible to most of the world population that does not have access to high-quality health care. It is almost certain that technological problems relating to accuracy of sequencing information will shortly be solved; however, the same is not true for the challenges in interpretation.

Although a detailed discussion of interpretation paradigms deserves detailed scholarly study and robust discussion among basic sciences, clinicians and policy makers, it is important to highlight some key points. The scientific community has heavily focused on the sequencing of phenotypic extremes, derived models of genetic architecture and allelic causality from these extremes, and is now seeking to superimpose these models on the general population. Given that we have at present a poor understanding of the effect of individual alleles that are superimposed on the genetic context of the rest of the genome, these assumptions are premature. We now understand that each individual can carry dozens of nonsense mutations, some of which appear to lie in genes thought to be crucial to biological function[66]. However, discarding such alleles from clinical relevance could be fundamentally flawed in the context of other alleles, epialleles and environmental exposures. Likewise, we are troubled by the flaws in the approaches to sequencing for prenatal defects from maternal fetal blood as a guiding tool, as such efforts are still grounded on a narrow view of genetic causality. It is important to stress that, given our limited ability to predict phenotypic outcomes on the basis of the genotype, offering pre-emptive guidance might be catastrophic. From our own work, we understand that patients bearing the M390R allele in *BBS1* may have no phenotype, may develop isolated retinal degeneration or may experience the full spectrum of Bardet–Biedl syndrome. Finally, variable penetrance and variable expressivity remain acute problems in clinical management and interpretation, the genetic basis of which must be understood more fully to improve the clinical utility of WGS data[143,144].

We strongly encourage the systematic study of both patient and control populations wherein genomic data are systematically annotated with detailed clinical information and physiologically relevant biological assays. We propose that these activities will be necessary to gain a sufficient understanding of the genetic architecture of human pathology and to improve the validity of computational prediction algorithms to the point at which their implementation in the clinical setting can be executed with confidence.

Finally, amid the discussion of what information should be delivered and how, we must be diligent to avoid genetic exceptionalism and threatening paternalistic approaches. Rather, we should work on bilateral communication mechanisms and policies that facilitate the exchange of annotated genetic information, accompanied by lucid assessment of the shortcomings and risks of such data, between clinical laboratories and patients.

Epialleles
An epigenetic variant of an allele. The activity of an epiallele is dependent on epigenetic modifications such as histone deacetylation or cytosine methylation.

Genetic exceptionalism
The view that genetic information, traits and properties are qualitatively different and deserving of exceptional consideration.

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Competing interests statement
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FURTHER INFORMATION
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ClinVar: http://www.ncbi.nlm.nih.gov/clinvar
DECIPHER: http://decipher.sanger.ac.uk
Duke Center for Human Disease Modeling: http://www.cellbio.duke.edu/CHDM/Home.html
Duke Task force for Neonatal Genomics: https://www.dukegenes.org
Genetic Testing Registry (GTR): http://www.ncbi.nlm.nih.gov/gtr
GeneTests: http://www.ncbi.nlm.nih.gov/sites/GeneTests
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Human Gene Mutation Database (HGMD): http://www.hgmd.cf.ac.uk/ac/index.php
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