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Clinical application of next-generation sequencing to the practice of neurology

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Abstract

Next-generation sequencing technologies allow for rapid and inexpensive large-scale genomic analysis, creating unprecedented opportunities to integrate genomic data into the clinical diagnosis and management of neurological disorders. However, the scale and complexity of these data make them difficult to interpret and require the use of sophisticated bioinformatics applied to extensive datasets, including whole exome and genome sequences. Detailed analysis of genetic data has shown that accurate phenotype information is essential for correct interpretation of genetic variants and might necessitate re-evaluation of the patient in some cases. A multidisciplinary approach that incorporates bioinformatics, clinical evaluation, and human genetics can help to address these challenges. However, despite numerous studies that show the efficacy of next-generation sequencing in establishing molecular diagnoses, pathogenic mutations are generally identified in fewer than half of all patients with genetic neurological disorders, exposing considerable gaps in the understanding of the human genome and providing opportunities to focus research on improving the usefulness of genomics in clinical practice. Looking forward, the emergence of precision health in neurological care will increasingly apply genomic data analysis to pharmacogenetics, preventive medicine, and patient-targeted therapies.

Introduction

The contribution of genetic factors to neurological diseases has long been recognised, and many successes of early molecular genetic technologies were in identifying genes associated with neurological disorders.[1] Of around 20 000 different genes in the human genome, more

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Declaration of interests

We declare no competing interests.

For the **Online Mendelian Inheritance in Man catalogue** see https://www.omim.org/

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than 80% are actively expressed in the brain.[2] Of more than 5000 genetic disorders with a known molecular basis that have been described, approximately 40% involve the brain or the nervous system, as documented in the Online Mendelian Inheritance in Man catalogue. However, until the advent of next-generation sequencing, phenotypic and genotypic heterogeneity[3–8] and low-throughput technologies for genetic testing (primarily Sanger sequencing) meant that most patients did not receive a molecular diagnosis, even if they had disorders with undisputed genetic aetiology. Consequently, only vague diagnostic descriptors (ie, developmental disorder, leukodystrophy, or polyneuropathy) were used, and patients received little prognostic information, no calculations of risk or recurrence for their family members, and no prospect of therapies being developed on the basis of an understanding of disease mechanisms. Roughly a decade ago, next-generation sequencing technologies came into use[9,10] and their effectiveness was quickly tested for diagnosing neurological disorders.[6,11,12] Since the inception of exome sequencing as a clinical diagnostic test, it has been routinely used for patients suspected to have rare Mendelian disorders.[13–15] Many laboratories have also started to introduce gene-panel or targeted sequencing tests using next-generation sequencing and to move away from Sanger sequencing, quantitative PCR, and multiplex ligation-dependent probe amplification, as next-generation sequencing is more cost-effective,[16] streamlined, and allows easy expansion of the number of target genes analysed. The technology has not only enabled much wider and more rapid clinical diagnostics,[6,12,17–33] but has also helped researchers to identify multiple previously unrecognised neurogenetic disorders.[8,34]

Genomic data are becoming more widely used in routine health care, not just in neurology but in all branches of clinical medicine; for example, to detect hereditary forms of cardiac disease, connective tissue disorders, endocrine conditions, or metabolic diseases.[35,36] Thus, the use of technologies capable of surveying a patient’s entire genetic landscape for critically relevant clinical information has naturally given rise to personalised medicine and precision diagnostics. These achievements in turn could contribute to improved clinical outcomes and could lead to genetically targeted therapies. In this Review, we outline the successes and challenges in the use and implementation of these technologies, and the potential changes as the next wave of genomic medicine begins.

Whole exome sequencing

Whole exome sequencing analyses the protein-coding portion of the genome (about 1–2%), where most disease-causing mutations identified to date are found.[13–15] This technique is particularly efficient at identifying such mutations in phenotypically variable conditions and has been tested and used for the molecular diagnosis of virtually all neurological phenotype categories, such as intellectual disabilities and other neurodevelopmental disorders, cerebellar ataxias, and epilepsies (figure 1).[6,12,17–33] Exome sequencing can directly confirm any of more than 5000 phenotypically and genetically diverse conditions with a single test, exceeding all other currently available clinical tests in diagnostic power. When disease phenotypes that are undisputedly genetic are properly identified, validated, and then analysed using exome sequencing, a molecular diagnosis can be established in about 40% of patients,[37] although for some disorders the proportion can be as high as 94%.[38] Exome sequencing is widely regarded as the current technology of choice for diagnosing monogenic

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neurological disorders, generally in combination with other technologies for detecting copy number variants, such as chromosomal microarray analysis, although, as we will consider in this Review, whole exome sequencing and chromosomal microarrays could soon be super ceded clinically by whole genome sequencing.

Variant identification and filtering

Unlike traditional molecular sequencing tests, the initial processing of next-generation sequencing data (appendix)[14] requires computationally sophisticated bioinformatic analysis to align target sequences to reference sequences in the human genome, regardless of whether the test encompasses only a few genes (targeted capture), the exome, or the genome. The human reference genome is a digital database of sequences from 13 anonymous donors from Buffalo, NY, USA.[39] It does not represent what would be considered a typical genome but instead represents the sequence and chromosomal location data for most of the human genome. It is therefore not complete and requires continuous updating to improve its accuracy.[40] Patient and reference sequences are aligned for comparison, any deviations from the reference genome are noted, and a list of these variants is generated. Early analysis of next-generation sequencing data showed that the human genome was vastly more variable than expected, necessitating interpretation and then filtering of these sequence variations.[41] Up to 30 000 variants can be detected in an exome analysis, and a typical genome sequence contains approximately 3–4 million variants.[42,43] Next-generation sequencing data must be filtered using a series of computational steps, termed bioinformatic pipelines, and manually curated to obtain a list of variants that are most likely to be clinically significant for further analysis.

The initial steps of filtering sequencing data partly depend on the premise that in healthy control populations there will be few or no pathogenic mutations. Therefore, the list of variants derived from a patient is bioinformatically annotated, particularly with information regarding the frequency of the variants in the general population, so that common variants are removed from analysis.[44,45] The minor allele frequency is a metric of an allele's rarity in a population and can be set at specific thresholds. Polymorphisms with a frequency greater than 1% are typically considered to be common and not likely to cause disease, whereas rarer variants (minor allele frequency <0·1%) are often considered more likely to be pathogenic. However, this distinction is somewhat arbitrary and exceptions are increasingly recognised where deleterious variants have high minor allele frequencies or benign variants have low ones.[46,47]

Because of the importance of considering such variation in the clinical interpretation of results, the Exome Aggregation Consortium (ExAC) has constructed a publicly available database of anonymous exome sequence data from more than 60 000 individuals.[45] Sequences from those people with early-onset disorders have been removed whenever possible. Data also include the frequency of homozygotes for each variant, as well as various constraint scores that indicate the tolerance of each gene to different types of variation on the basis of the comparison of observed frequencies to statistical expectations. The ExAC browser can be searched by gene and by specific genomic or protein variants to find minor allele frequency data. More than 120 000 exomes (including most ExAC data) and 15 000

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genomes have been deposited in the related Genome Aggregation Database (gnomAD).[45] These numbers are expected to grow, further increasing the usefulness of these resources. An important caveat is that both ExAC and gnomAD comprise only unrelated adult samples, which excludes severe paediatric-onset dis eases,[45] but can still include less severe, late-onset, or low-penetrance disorders. Two major additional limitations of both ExAC and GnomAD are that no individual phenotypes are available, and the ethnicity of the patients is highly skewed towards those of European descent (appendix). There are major differences based on ethnicity in some regions of the genome,[48] including enrichment of certain variants in specific populations, and this deficiency urgently needs to be rectified. Finally, it is important to emphasise that benign variants might also be rare or individual, and for this reason a variant with a low minor allele frequency should not be automatically considered as potentially pathogenic simply because of its rarity.

Several other gene databases used in clinical practice can assist in rapidly determining the probable pathogenicity of previously reported genetic mutations. The Human Gene Mutation Database[49] and ClinVar[50] are the two most commonly used databases to annotate variants as disease-causing on the basis of previous observations in affected individuals (appendix). However, because these databases are primarily constructed from literature searches using data predating next-generation sequencing technologies, misclassification of variants is not uncommon and more stringent methods to classify variants are now recommended.[51]

Disorder-specific databases that can assist in variant interpretation by enabling comparison of phenotypic data with genotypic information on a massive scale are also becoming available. The Deciphering Developmental Disorders collaborative study[52–55] based in the UK and Ireland studied more than 13 000 children with undiagnosed developmental disorders using high-resolution genome-wide single nucleotide polymorphism arrays and trio-exome sequencing. Some of the data are publicly available through the DECIPHER database and includes information on probable pathogenicity based on in-silico and clinical analysis. There are also other disease-specific databases for neurological disorders, such as the DMD mutations database by UMD-DMD France and the Leiden Muscular Dystrophy pages, which can assist with variant interpretation. Best practice of next-generation sequencing interpretation in clinical practice requires liaison with both clinical geneticists and molecular diagnostic laboratories.

Clinical interpretation of gene variants

Interpretation of next-generation sequencing data is subtly but importantly different between clinical pract ice and research settings. The fundamental question in clinical practice is whether a gene variant (or variants) adequately explains the patient’s phenotype and, crucially, whether the data can be used for further genetic counselling or other clinical management (panel). The reporting standards must be rigorous and stringent because incorrect interpretation and reporting has disastrous consequences for individuals and their families.[51] For example, in the field of cancer genetics, wrong interpretation has led to unnecessary surgical interventions or inappropriate screening strategies.[62] Thus, in routine clinical practice, only variants in genes that are already causally associated with disease and

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considered to be pathogenic on the basis of predefined criteria, of which examples follow, are routinely reported.

Interpretation often commences with assessment of variant frequency. However, heavy reliance on interpreting data using allele frequency could be misleading. For example, extensive clinical and functional data support a role of *SPG7* mutations in hereditary spastic paraplegia, and evidence from next-generation sequencing studies supports its fundamental role in spastic and pure cerebellar ataxia.[6,63] One specific *SPG7* variant (1529C→T; Ala510Val) could be interpreted as a benign polymorphism on the basis of its frequency in control populations. GnomAD contains 820 alleles with this variant of the 282 858 alleles in the database, including two homozygotes, showing that data from patients with late onset of disease can be present in such databases. However, evidence[64] suggests that this variant is pathogenic, although clinical laboratories have been unable to reach a consensus on this interpretation.[50,65] Clearly, additional research is needed to understand whether this is an allele with reduced penetrance in the general population and, if so, what are the implications for conveying these results to carrier families. A similar issue has been reported for *NOTCH3*, mutations in which cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). ExAC contains an unexpectedly high frequency of variants in *NOTCH3* associated with CADASIL, suggesting that some variants have reduced penetrance, which has substantial implications for genetic counseling.[57] These reports show that caution is required in using such data in clinical practice without considering the evidence in its entirety, especially if life-changing actions are made on the basis of the test results.

Interpretation of variants tends to be simplified for well known, disease-associated genes with established data on pathogenicity using these population and mutation databases. In-silico predictive pathogenicity programs, such as Sorting Intolerant from Tolerant (SIFT), PolyPhen2, Mutation Taster, MutPred2, Variant Effect Predictor (VEP), SNPEff, or Combined Annotation Dependent Depletion (CADD)[66] can help with variant interpretation, particularly of missense mutations. These tools are based on various parameters, such as the structural or biochemical effects of an amino acid substitution and the conservation of a nucleotide or amino acid residue. However, the results of such computer-based algorithms can vary substantially, with contradictory interpretations[67–70] compounded by the complexities of reduced penetrance, variable expressivity, mosaicism, epigenetics, modifying genes, or environmental influences.[71,72] Therefore, programs for pathogenicity prediction are best used to provide supporting evidence to clinical and other data.[44]

Additional information to assist in variant interpretation can include familial segregation data, particularly in larger families with multiple affected individuals. This can be done at the start of sequencing (eg, trio sequencing of proband and parents), or post hoc in individual cases, only analysing plausible variants. Trio sequencing can rapidly exclude irrelevant variants, even if they are rare.[22] The most notable successes of this type have been in identifying de-novo variants in a wide variety of neurodevelopmental disorders, including early-onset epilepsy syndromes, intellectual disabilities, autistic spectrum disorder, and some ataxia syndromes.[73,74] However, in smaller families, this method might still prove inconclusive, particularly in cases of apparent de-novo inheritance, since not all such

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variants are pathogenic, and other factors that influence the inheritance pattern (eg, germline mosaicism in a parent)[75] might be difficult to detect if sequencing in blood is uninformative.

The difficulties of interpretation due to the volume of next-generation sequencing data becoming available and concerns about over-interpretation of variants led the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) to publish a series of consensus guidelines on variant interpretation for clinical practice.[44] These guidelines incorporate detailed information on the mutation's phenotype, mode of inheritance for the disease gene, mutational mechanism (ie, haploinsufficiency, dominant negative, loss-of-function), protein structure and function, and evidence of the gene–disease relationship based on available literature and population data. Variants are classified according to clear rules as "pathogenic", "likely pathogenic", "uncertain significance", "likely benign", or "benign" on the basis of this information.[44] The authors of these guidelines note that more genetic changes are likely to be classified as "variants of uncertain significance" (VUS; eg, a variant where bioinformatic analysis in patients or the general population cannot reach a conclusion on disease pathogenicity) on the basis of these guidelines. However, they also note that this classification is balanced against the potential harm that might otherwise result if these variants are reported as pathogenic because of insufficient evidence.

In cases where a variant cannot be interpreted as causative for a particular disease, its definitive classification might require detecting it in other affected individuals and additional functional research testing. Reanalysis of VUS or otherwise negative exomes at regular intervals could be useful as new disease genes are identified and variants become reclassified over time on the basis of new evidence.[13–15,55,76–78]

Multidisciplinary team approach to analysis of variants

Ultimately, the decision on how to classify each variant is made through consensus interpretation and is beyond the training and expertise of most clinicians. It is becoming more common for physician training programmes to include discussions of genomic data and their interpretation, and there are increasing opportunities for postgraduate education in this field. Many centres have developed multidisciplinary teams who meet on a regular basis to discuss the interpretation and reporting of next-generation sequencing genomic variants.[22,79,80] These teams can comprise laboratory clinical scientists, bioinformaticians, clinical and research geneticists, neurologists, pathologists, genetic counsellors, and other clinical specialists. Whenever possible, disease-specific or phenotype-specific specialists should be involved in these discussions. However, classification might still vary,[65] as interpretation of the ACMG/AMP guidelines and resources used for classification differ among laboratories, along with the availabilty of detailed clinical information. Re-evaluation of variants on a regular basis with updated resources, collaborative efforts to deposit accurate information in public databases, and physician education to interpret reported variants in the context of a patient's phenotype are essential for accurate classification. The ACMG/AMP guidelines have proven helpful in assisting such teams to improve variant interpretation, for example by recommending review or additional characterisation of a patient's phenotype or specific additional diagnostic studies. For example, a referral request for epilepsy might not be useful

for interpreting variants in a gene reported to cause myoclonic epilepsy, but might prompt a detailed review of the phenotype (and possibly additional investigations) that corroborate or refute this diagnosis to assist in the variant's reclassification as either pathogenic or benign.

Discovery of novel disease genes

Variant interpretation in clinical practice must be stringent and generally exclude reporting of genes for which there is currently no known clinical relevance. However, when new candidate genes are identified, it is essential that their association with disease is validated and transitioned into clinical practice as soon as possible. For many neurological phenotypes (ie, cerebellar ataxia, amyotrophic lateral sclerosis, polyneuropathies, intellectual disabilities), the number of disease-associated genes identified has strikingly increased since the introduction of next-generation sequencing.[34] An increasing challenge for studying rare diseases is that variants might be identified in genes affecting only a few or individual families, making it harder to fulfil the required burden of proof that these variants are pathogenic. As we have already described, large databases can be helpful; for example, there are more than 100 publications since 2011 reporting newly defined genetic syndromes based on data submitted to the Deciphering Developmental Disorders database. Addition ally, there are ongoing collaborative efforts to link clinical and research groups that have identified rare variants in the same genes.[81] One of the most commonly used databases for this collaboration is GeneMatcher,[82] an online tool specifically built to advance novel gene discovery in rare diseases.[81]

The role of functional studies in identifying disease-associated genes

Analysis of the functional effects of genetic variants is rarely used in clinical practice. In research settings, however, providing extensive evidence of a variant's pathogenicity through functional studies is typically a prerequisite to verify that it causes disease. A common method is testing the effect of a genetic variant in model systems, including various organisms or patient-derived cultured cells. Gene-specific or biochemical assays can also be used. Research using functional studies is important for clinical practice because it can validate newly described disease-associated genes and support the interpretation of specific variants. Functional data comprise an essential part of the ACMG/AMP guidelines for variant interpretation.[44] Although such strategies do not perfectly reproduce human disease and are subject to technical and other caveats,[83] it is very probable that they will be used more frequently in future studies to improve the accuracy of rare variant interpretation and validation.[70]

Limitations to whole exome sequencing

In many neurological phenotypes, molecular diagnosis is consistently less than 40%, and it is important to consider the underlying reasons. Some patients thought to have genetic disease might ultimately have an identifiable acquired cause, particularly in sporadic cases, given the well recognised phenotypic heterogeneity that exists between genetic and acquired aetiologies in neurological disorders.[13] Beyond that, there are many reasons why exome sequencing might not identify a genetic cause. Some are technical, generally due to incomplete or nonuniform depth of sequencing coverage, sometimes due to low capture efficiency, or because of genomic regions that are difficult to sequence, such as GC-rich

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regions, highly repetitive elements, and pseudogenes. Furthermore, some variant types, such as repeat expansions, complex copy number variants, or other variants causing structural abnormalities in the genome, are challenging to identify, requiring specialised testing or additional bioinformatic pipelines that are still in development.[84] Mutations occurring in non-coding sequences and somatic or germline mosaicism will typically not be detected. Interpretation pipelines might differ in their stringency of variant interpretation for the target clinical population. Finally, insufficient clinical information and the inability to identify all known disease-associated genes might be the most common reasons hindering diagnosis. Specific disorders for which the usefulness of exome sequencing is still unproven include Parkinson's disease, Alzheimer's disease, and cerebrovascular disease. However, even in these conditions, if patients are carefully selected according to phenotype, age of onset, and family history, next-generation sequencing can still potentially be valuable in separating out conditions due to Mendelian disorders and assessing variation associated with genetic risk.

Gene panels based on next-generation sequencing

To maximise efficiency and minimise cost, gene panel testing has been used to focus attention on those genes most often associated with a specific disease or key phenotype. Initially, this approach included targeted capture of the regions of interest followed by sequencing. Subsequently, an alternative and increasingly prevalent strategy has been to do panel testing using filtered exome sequencing data—ie, the whole exome is sequenced but only the genes in a specific, laboratory-defined panel are analysed. An advantage of this method is its improved cost-effectiveness, since a pathogenic mutation can be identified quickly in a known gene and fewer VUS requiring review will be identified. This approach also gives flexibility to the laboratory to expand the panel as needed if the initial analysis is negative or if new candidate genes are identified.[18,42] From an interpretative standpoint, the approach might alleviate the challenges of analysing variants identified in clinically irrelevant or phenotypically unrelated genes. However, this method might report more VUS in the tested genes and can be further misinterpreted because of confirmation bias, varying expressivity, and phenotypic heterogeneity.[15] A consensus might also be absent regarding which genes should be included in a panel. Furthermore, exome data tend to have less uniform sequence coverage than targeted capture or genome sequencing, so genes of specific interest might need to be gap-filled using Sanger sequencing. Another alternative is to do panel analysis on genome sequence data. This is an increasingly cost-effective strategy as sequencing becomes cheaper, and is currently being trialled in the UK 100 000 Genomes Project.

Whole genome sequencing

Some clinical diagnostic laboratories have begun offering whole genome sequencing, instead of or in addition to, exome sequencing. A typical whole genome has approximately 4·5–5·0 million single-nucleotide and insertion-deletion variants per sample.[41,43] After filtering out common and probably benign variants about 400 000 typically remain for interpretation in the context of the clinical indication and it is impractical to analyse such a large number of variants.[41] For this reason, the interpretation and reporting of variants from whole genome sequencing are still almost exclusively restricted to coding regions.

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Whole genome sequencing can be very powerful when applied to studies of multigenerational families[85–87] or when coupled with other measures of gene dysregulation, such as transcriptome analysis.[88] Therefore, gene panels or targeted sequencing based on next-generation sequencing techniques or whole exome sequencing are still more common in clinical practice. However, as sequencing costs decline and interpretation pipelines for non-coding regions improve, this strategy is likely to change. The value of whole genome sequencing in neurological practice is being investigated as part of the 100 000 Genomes Project, the largest study of its kind, designed to leverage genomics to improve patient care. There are many theoretical advantages of whole genome sequencing compared with whole exome sequencing, although there are only a few studies[89–91] directly comparing the two for the purpose of clinical diagnosis. Such advantages include the facts that structural variants, along with copy number variations, can be identified with higher sensitivity and better resolution with whole genome sequencing than with whole exome sequencing or chromosomal microarray analysis, and that these variants can be mapped to a precise chromosomal location.[92] Other advantages include more uniform depth of coverage than exome sequencing, and the potential ability to detect expanded repeats (eg, short tandem repeats), which are common causes of neurological disorders such as Huntington's disease or amyotrophic lateral sclerosis.[93]

Despite such advantages, most of the rare variants detected with whole genome sequencing are within intergenic regions, mostly in the untranslated region or introns, and are thought to contribute to altered regulation of gene expression, which is difficult to assess without functional testing. One method to functionally interpret these non-coding variants involves complementing the sequencing data with transcriptome data (ie, via sequencing of RNA generally extracted from blood, skin, or muscle) to detect changes in RNA splicing, allele-specific expression, or differential expression (appendix).[94,95] This additional testing could greatly improve the diagnostic value of whole genome sequencing, as studies have shown that synonymous variants and deep intronic variants can result in splicing and other RNA processing defects.[94,95] Some commercial laboratories are already beginning to offer transcriptome analysis as a commercial test to complement sequencing analysis. However, such genetic changes can only be detected when using an appropriate tissue type for the gene and disease of interest. Databases are now available that provide information on the tissue distribution and abundance of specific gene transcripts across the whole genome.[96] Since blood is the most frequently obtained tissue and genes of the nervous system do not follow the same expression patterns in lymphocytes as in brain or nerve,[96–98] research methods such as differentiating patient-derived induced pluripotent stem cells or skin fibroblasts into neuronal cells could eventually be required to overcome this challenge.

Considerable progress is already being made to annotate non-coding transcripts and gene regulatory domains, often in a tissue and cell-type specific manner.[99–104] By contrast with gene-coding mutations, non-coding mutations that disrupt important regulatory domains have the potential to influence pathways that govern cellular development and differentiation. For example, in a 2018 report,[105] a rare DNA translocation seen in a patient with microcephaly, polymicrogyria, and agenesis of the corpus callosum was found to disrupt a DNA enhancer region that participates in brain development. In another example, [102] schizophrenia risk genes and pathways were identified that involve non-coding DNA

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regulatory regions, suggesting that they might participate in the evolution of human psychological and cognitive function, which might also be true for RNA regulatory elements.[106] Ever-increasing research publications support the possible roles of non-coding mutations in causing genetic diseases, including neurological disorders.[85,107–109] As the number of individuals whose genomes have been sequenced increases and research efforts to functionally understand the genome continue to advance, the ability of clinicians to apply whole genome sequencing to diagnose disease will also improve.

Ethical considerations

The ability to obtain personal genome-wide sequence data raises many ethical issues, which must be addressed during counselling before and after the test. Counselling must include a discussion of the test modality, the clinical and health implications of a molecular diagnosis (or lack thereof), and the concept of secondary findings. Other points of discussion are the role of parental samples in test interpretation, consanguinity, and the possibility of identifying non-parentage. In some clinical settings, addressing insurance implications, authorisation, and test costs might be appropriate. Because these issues are complex, they are often addressed in a clinical genetics setting and formal written consent is generally advised before testing.

Secondary findings

Occasionally, patients who undergo genetic testing are found to have mutations in genes that are unrelated to the disease that prompted the initial evaluation. Such occurrences were well recognised before the introduction of next-generation sequencing into clinical practice, but their frequency has increased because larger amounts of genetic material are now being analysed than previously. The classic illustrative example is where a dominant pathogenic or predisposing cancer mutation in a gene such as *BRCA1* is identified. To address this concern, the ACMG/AMP have published recommendations for reporting such findings for clinical evaluation.[110] However, diagnostic laboratories are not obliged to follow these guidelines or might modify them, for example to add additional genes, include carrier status for certain disorders, or report pharmacogenetic variants. Hence, the identification of such secondary findings can vary per cohort, ranging from as high as 5% to less than 1%.[22,31,32,111] Nevertheless, the consensus for all genomic tests is to provide pretest and post-test genetic counselling that includes a discussion of secondary findings and documents the patient’s preference of how such results are reported.[112,113]

Direct-to-consumer genomic testing

Many companies now offer various services, ranging from exploring heredity, through screening for common disease risk variants, to searching for rare disease-causing mutations.[114–116] However, these companies do not operate within standard health-care environments, and the consent processes and quality of results might not conform to the stringency required in clinical practice.[117,118] Consequently, patients whose family members undergo direct-to-consumer genomic testing might find themselves (and possibly their children or other relatives) inadvertently faced with issues of heredity, paternity, carrier status, or

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presymptomatic mutations. These considerations must be clearly addressed when counselling patients before diagnostic testing.

Genetic risk

In addition to single-gene Mendelian inheritance, there is ample evidence for gene variants conferring risk of disease due to variable alterations in cellular function, sometimes modulated by other genes or epigenetic and environmental cues.[119–121] Consequently, many variants exist in the population with minor degrees of potential influence on disease. Alone, they might not be enough to cause disease in most circumstances. Rather, they probably affect health by altering the risk of sporadic disease, in combination with other factors. One of the best known genetic risk variants is *APOE* ε4 in Alzheimer's disease.[122] Carriers of a single copy of the *APOE* ε4 allele are roughly 2·5 times more likely to develop Alzheimer's disease than non-carriers.[122,123] Direct-to-consumer or commercial testing that reports the presence of this or similar polymorphisms tends to describe them as increasing the risk of disease. However, such information is typically inadequate to determine if a specific individual will develop the disease or not[124] and provides insufficient information for clinical management. In most instances, despite an increased risk relative to the non-carrier population, the absolute risk of developing the disease might still be very low. Nevertheless, the discovery of preclinical disease pathology in individuals who later develop neurodegenerative disorders such as Alzheimer's disease suggests that early recognition of those patients who are at highest risk could facilitate early therapeutic interventions, first through clinical trials and then with tailored preventive strategies.[125–129] Additional research is needed to realise the potential of linking strategies for genetic risk assessment to disease prevention and therapy (appendix).

Future directions: precision health

A molecular diagnosis in clinical neurology is central to guiding clinical management. For example, identifying intellectual disability caused by a *PTEN* mutation or ataxia due to a mutation in *ATM* could suggest that screening for specific forms of cancer, with which such mutations are also associated, is also necessary, whereas establishing a genetic diagnosis of adrenomyeloneuropathy or cerebrotendinous xanthomatosis might necessitate testing for specific endocrine dysfunction and subsequent treatment. In addition, there are ample data to support the use of next-generation sequencing in reducing the patient's time to diagnosis, often referred to as the "diagnostic odyssey" (appendix). Precision health encompasses the use of patient-specific data to tailor patient-specific care.[130,131] It addresses a fundamental deficit in previous approaches to diagnosing neurogenetic disease—genetic and phenotypic heterogeneity that is unaccounted for.[132] This heterogeneity often limits a clinician's ability to predict whether a particular therapy will be effective in a given individual, despite the drug in question having significant benefits in group analyses. Inability to address this heterogeneity is hypothesised to contribute to failed drug trials, preventable adverse effects, and inefficient use of available therapies that culminates in considerable monetary waste and minimal or no benefit to the patient or their families.[133–136] Patient-tailored therapies have the potential to create a major paradigm shift in medicine,[132] which is already happening in neurology (appendix), particularly in the field of neurometabolic disorders. These include

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glucose transporter type-1 deficiency (introduction of ketogenic diet),[137] X-linked adrenoleukodystrophy (bone marrow transplantation or gene therapy),[138,139] Brown-Vialetto-Van Laere syndrome (treatment with riboflavin),[140,141] and dopa-responsive dystonia (treatment with levodopa).[142] A study from the University of British Columbia[143] reported that genetic diagnosis altered the treatment of 44% of their paediatric patients with neurometabolic disease.

Precision approaches have also helped progress in the diagnosis and treatment of epilepsy syndromes. Common problems in refractory epilepsy include the challenges of trial-and-error drug selection that can result in undesirable polytherapy, seizure-related injury, side-effects, and cost.[144] In genetic epilepsy syndromes due to single-gene Mendelian mutations (about 1% of paediatric epilepsies), the efficacy of specific anti-epileptic drugs can be directly related to the underlying mutation, as is the case in Dravet syndrome, for which treating patients with sodium channel blockers is contraindicated.[145,146] On a research basis, there is considerable interest in clinical pharmacogenetics, where patient treatment could potentially benefit from the use of genomic data. For example, polymorphisms of *CYP2C9* might reduce the metabolism of phenytoin by 25–50% and have been associated with increased adverse events,[147] whereas the *HLA-B**15:02 polymorphism is associated with potentially toxic drug side-effects to phenytoin and carbamazepine in certain populations.[147,148] The application of pharmaco genetics to treatment and diagnosis extends beyond epilepsy and is a clinical area that is still in development.[149] Several pharmacogenetic profiling tests and services are clinically available to inform the likelihood of a patient's response or risk of developing side-effects from specific drugs, including certain psychiatric, cardiac, and pain medications. Over time, the use of patient genetic data to predict drug efficacy and minimise side-effects will probably expand as research into these areas progresses.

Conclusions

Next-generation sequencing has been firmly established as the technology of choice to rapidly and efficiently diagnose neurological disorders with a probable Mendelian genetic basis (figure 2). Efficient diagnosis will enable targeted therapies and better management. Widespread use of genomic information enables a precision-health approach to treat not only active disease in a specific patient, but to broadly envision the prevention of future disease (appendix). Complementary efforts are underway to incorporate large datasets, including those of patient genotypes, with clinical outcomes data to develop data-based predictors of therapeutic response and prognosis to common therapies across a variety of health conditions. Effective data-driven predictive algorithms are in development to merge large amounts of patient data and to integrate multiple clinical data types (eg, merging genomic sequence data directly with the electronic medical record for large-scale, disease-relevant search and discovery). A future could be possible where the clinician prioritises disease-modifying drugs in neurological diseases such as multiple sclerosis, epilepsy, or stroke by imputing patient-specific variables, including genotype, into a data-based algorithm and considering several options ranked by predicted efficacy and tolerability. With unprecedented amounts of human data being generated from patients and healthy individuals, coupled with major developments in technology and large-scale data analysis,

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advances in genomics and precision health are creating new opportunities for evidence-based and patient-centred care. The next decade promises major shifts in the translation of these technologies into the clinical setting that will certainly benefit patients with neurological diseases.

Supplementary Material

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See **Online** for appendix

For the **Exome Aggregation Consortium database** see http://exac.broadinstitute.org/

For the **Genome Aggregation Database** see http://gnomad.broadinstitute.org/

For the **DECIPHER database** see https://decipher.sanger.ac.uk/ddd#research-variants

For the **UMD-DMD France database** see http://www.umd.be/DMD/W_DMD/index.html

For the **Leiden Muscular Dystrophy pages** see http://www.dmd.nl/

For the **Sorting Intolerant from Tolerant database** see http://sift.bii.a-star.edu.sg/

For the **PolyPhen2 database** see http://genetics.bwh.harvard.edu/pph2/

For the **Mutation Taster database** see http://www.mutationtaster.org/

For the **MutPred2 database** see http://mutpred.mutdb.org/

For the **Variant Effect Predictor databse** see https://uswest.ensembl.org/info/docs/tools/vep/index.html

For the **SNPEff database** see http://snpeff.sourceforge.net/SnpEff_manual.html

For the **Combined Annotation Dependent Depletion database** see http://cadd.gs.washington.edu/

For the **GeneMatcher database** see https://www.genematcher.org/

For the **100 000 Genomes Project** see https://www.genomicsengland.co.uk/

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Panel:

Cases illustrating the clinical benefits of next-generation sequencing

Case 1

A 23-year-old man with a past medical history of developmental delay, intellectual disability, and autism spectrum disorder was evaluated for progressively worsening cerebellar ataxia occurring over 6 months. Neurological examination was notable for limb, trunk, and gait ataxia, as well as for diffuse hyper-reflexia. A detailed evaluation for acquired causes was unremarkable, although diffuse white matter changes were noted on brain MRI. Family history showed no known parental consanguinity, but both parents came from the same small town in Mexico. The patient had no previous genetic testing. Given the broad differential diagnoses for the phenotypes under consideration (eg, intellectual disability, autism spectrum disorder, and cerebellar ataxia), whole exome sequencing was done. A homozygous deletion causing a frameshift in the *CYP27A1* gene encoding sterol 27-hydroxylase was identified. The gene is associated with an autosomal recessive lipid storage disease known as cerebrotendinous xanthomatosis.[56] The typical presentation involves onset of tendon xanthomas during adolescence or early adulthood, with progressive neurological dysfunction including psychiatric disturbances, dementia, pyramidal signs, ataxia, parkinsonism, peripheral polyneuropathy, and seizures. Early cataracts and diffuse white matter changes on MRI are common. Cataracts or tendon xanthomas were not identified in this patient; however, the concentration of 5α-cholestan-3β-ol (cholestanol), a biomarker for the condition, was elevated (40 μg/mL [normal 0·9–3·7 μg/mL]), confirming the diagnosis. Treatment is with chenodeoxycholic acid, which bypasses the enzymatic block and corrects the metabolic abnormities. It was started immediately and the patient's neurological symptoms stabilised, preventing further damage to the nervous system.

Case 2

A 68-year-old woman with a history of migraine headaches with aura and transient ischaemic attacks was evaluated for a possible genetic syndrome. Brain MRI showed severe microvascular ischaemic changes in the cerebral and brainstem white matter, multiple old lacunar infarcts in the periventricular white matter, and old microhaemorrhages in the cerebrum and brainstem. Her mother, sister, and brother had a similar history of transient ischaemic attacks and her brother had a similarly abnormal MRI. Consanguinity was not reported. Multiple vascular neurology specialists had given the patient a clinical diagnosis of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL),[57] given that her phenotypic presentation was consistent with CADASIL; however, single-gene sequencing of *NOTCH3* was negative for coding variants and skin biopsy was negative for typical histopathology. Whole exome sequencing was done and identified a missense variant in *HTRA1* (Val279Met). Although loss-of-function variants in *HTRA1* are typically associated with autosomal recessive cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), other variants have been found to cause an atypical form of CADASIL, termed CADASIL2.[58–61] CADASIL2 was consistent with the clinical

presentation of this patient, resolving the diagnostic dilemma and enabling proper genetic counselling of the patient and her family.

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Search strategy and selection criteria

We searched PubMed through Jan 1, 2013, to Oct 31, 2018, using the terms “next-generation sequencing“, “exome“, “genome“, “precision health”, “diagnosis”, “utility”, “challenges”, “genomics”, and “neurogenetics” singly and in combination. Disease or gene names and phenotypic descriptors were added to the search as appropriate. Articles were also identified by the authors from their own field of study, as well as from the references of relevant articles. Because of space limitations, emphasis was placed on articles published after 2013. Only papers published in English were considered.

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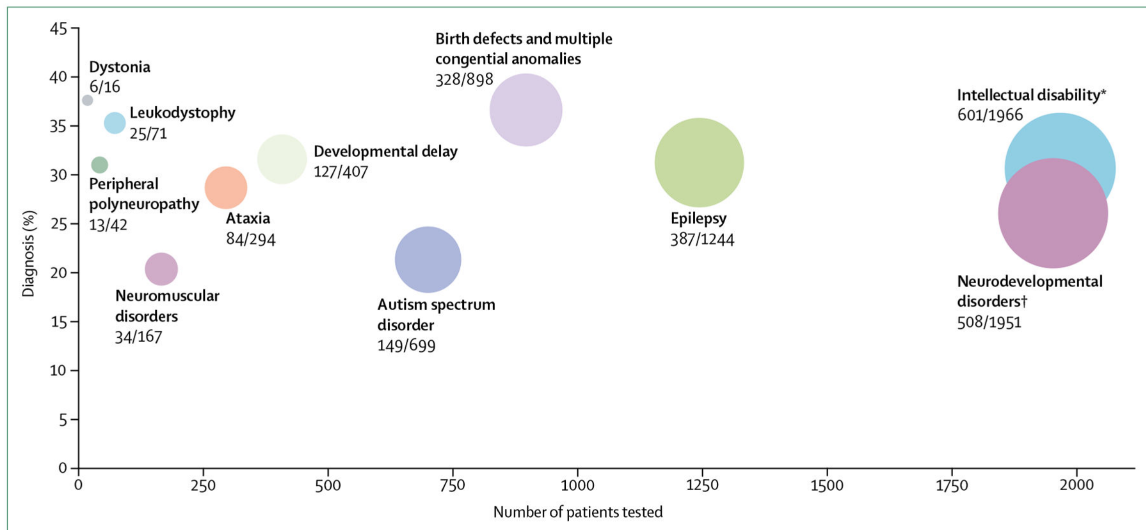

Figure 1: Proportion of patients molecularly diagnosed with various neurological diseases by whole exome sequencing

The percentage and ratio of patients with a positive genetic diagnosis is indicated for a variety of neurological diseases tested by whole exome sequencing. The total number of patients whose exomes were sequenced is indicated on the X-axis for each neurological disorder and reflected in the relative size of each data point. Studies[6,12,17–33] from which data were obtained were included on the basis of the disease under investigation and whether exome sequencing was done in the context of a clinical diagnostic test. Studies exclusively using next-generation sequencing panels or partial exomes were not included. *Might include developmental delay. †Includes developmental delay, intellectual disability, and autism spectrum disorder.

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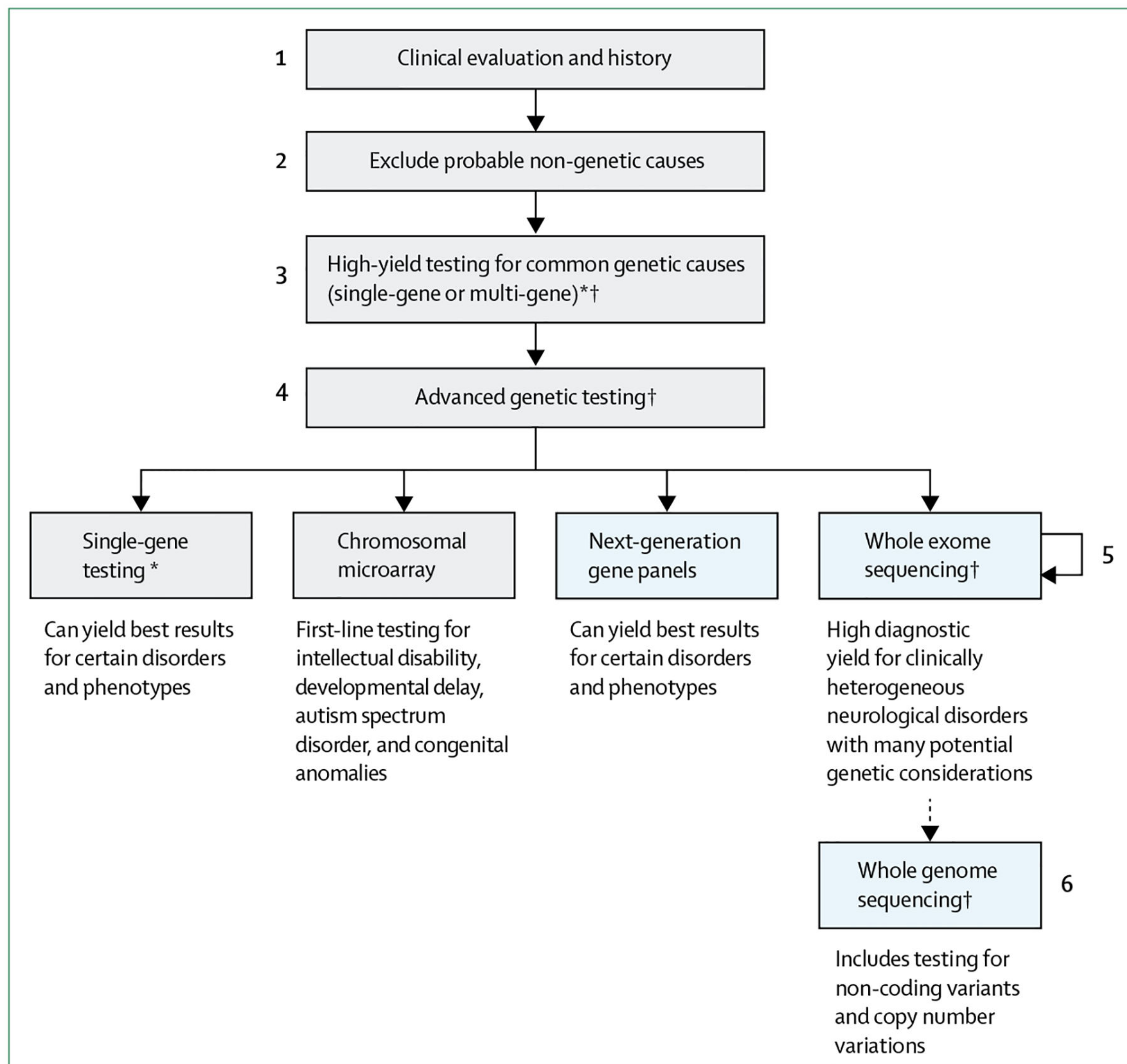

Figure 2: General strategy for incorporating next-generation sequencing into the diagnostic evaluation of a patient with suspected neurogenetic disease

A generic workflow for a diagnostic genetic assessment incorporating next-generation sequencing (blue) and specifically whole exome sequencing is proposed, according to the following sequence: (1) establish phenotype; (2) confirm indication for genetic testing; (3) high-yield testing done on the basis of phenotype or if the most common genetic causes are not detectable by sequencing (eg, nucleotide repeat expansion, deletion, duplication, etc); (4) if advanced genetic testing is necessary, consider the specific disorder or phenotype to determine the most appropriate test; (5) if no diagnosis is achieved with whole exome sequencing, consider regular clinical and bioinformatic re-analysis; after which (6) consider whole genome sequencing for any future analysis, if available. *If considering high-yield single-gene testing of more than 1–3 genes by another sequencing method, note that next-generation sequencing is often most cost-effective. †Genetic counselling is required before and after all genetic testing; other considerations include the potential for secondary findings

in genomic testing, testing parents if inheritance is sporadic or recessive, and specialty referral. Modified from Fogel,[13] by permission of Elsevier.

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