REVIEW ARTICLE

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Genetic Diagnostics for Neurologists

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ABSTRACT

PURPOSE OF REVIEW: **This article puts advances in the field of neurogenetics into context and provides a quick review of the broad concepts necessary for current practice in neurology.**

RECENT FINDINGS: **The exponential growth of genetic testing is due to its increased speed and decreasing cost, and it is now a routine part of the clinical care for a number of neurologic patients. In addition, phenotypic pleiotropy (mutations in the same gene causing very disparate phenotypes) and genetic heterogeneity (the same clinical phenotype resulting from mutations in different genes) are now known to exist in a number of conditions, adding an additional layer of complexity for genetic testing in these disorders.**

SUMMARY: **Although the growing complexity of technical knowledge in the ordering and interpretation of genetic tests makes it necessary for neurologists to consult medical geneticists, limitations in the availability of such professionals often means neurologists will be on the front line dealing with suspected or confirmed neurogenetic conditions. The growing availability of broad genetic testing through chromosomal microarray and next-generation sequencing and the expanded phenotypic spectrum of many conditions has implications for genetic counseling and medical management. This article discusses the various forms of genetic variability and how to test for each of them. It also provides an update on the most common forms of neurologic presentations of genetic disease and a review of testing strategies.**

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INTRODUCTION

Advances in genetic testing technologies over the past 2 decades have revolutionized our ability to make specific molecular diagnoses in patients presenting with neurologic illness. Although this has brought a wealth of information and has already influenced the medical management of many neurologic conditions, it also presents a challenge for the practicing neurologist to stay updated in the rapidly evolving field of neurogenetics.

With the understanding of the surprising scope of variation inherent in the human genome, new technologies have come to the fore that allow clinicians to diagnose—and increasingly treat—entire new classes of neurologic disease. With this has come a new vocabulary, and while the basic concepts of human genetics

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may not have changed over the decades, the way we talk about these concepts and their relevance to clinical medicine have. The goal of this article is to introduce these concepts, together with the new vocabulary, in the context of practical approaches to the use of genetic diagnostic technologies in the care of our patients.

NEUROLOGY AND MEDICAL GENETICS

While neurologists will see and care for patients with genetic conditions with regularity, an entire field of medical genetics exists for a reason. Most neurologists have no training in genetic diagnostics. Some will be familiar with the more common neurogenetic conditions (mainly those within their field of subspecialty) or with specific genetic conditions they have encountered before. Neurologists should not feel pressed to embark upon genetic diagnostic evaluations with little ability to explain what results they expect to find and how to interpret the results when they do arrive. Neurologists do not expect geneticists to determine who needs an EEG or EMG, much less to interpret the results. Yet, neurologists routinely feel they need to marshal themselves to explore the genome, sometimes without a road map or compass and often without speaking the local language. Ideally, consultation with a geneticist should be actively pursued when suspecting or managing a neurogenetic condition, not only to prevent an illogical sequence of often expensive unnecessary diagnostic evaluations but also to avoid missing the correct diagnosis or appropriate management of patients. But, if a shortage of properly trained neurologists exists, as occurs in many regions of the world, the shortage of geneticists is usually even more acute, and many parts of the world may not have enough medical geneticists for the foreseeable future. This article cannot solve this problem but tries to mitigate it by providing neurologists with basic tools to communicate with colleagues, quickly get updated on conditions, and discuss with patients the need for genetics referral and testing.

One essential component involved in all genetic testing is genetic counseling. While standards of training for genetic counselors vary widely in different countries, even in developed nations, underserved areas exist in which the services of a genetic counselor are not available and either the neurologist or the medical geneticist will perform pretest and posttest counseling. Benefits, limitations, and potential complications of genetic testing should be discussed with patients, parents, or guardians, and written informed consent should be obtained for specific tests ordered. The benefits of genetic testing may include directly impacting medical management strategies, providing specific information on recurrence risk and reproductive counseling, providing labels that enable access to services, and giving access to educational material and support groups. Limitations of the specific test should be made clear, including the fact that more targeted testing may miss various differential diagnoses, while broader testing strategies (eg, chromosomal microarray, exome sequencing) increase the likelihood of unexpected or unclear results. Incidental findings may have an impact on family planning, medical screening, and medical management, and patients should be given the opportunity to waive the disclosure of these results.

The direct impact of genetic results on medical management includes preventing unnecessary further tests (including invasive and expensive tests), the organization of appropriate investigations and referrals for potential comorbidities (eg, specific ophthalmologic, cardiologic, and orthopedic complications that are frequent in neurogenetic conditions), targeting of medical therapies based on the genotype, and establishing eligibility for participation in

KEY POINTS

- **The growing complexity of technical knowledge involved in ordering and interpreting genetic tests makes the opinion of a medical genetics specialist desirable in the many cases of suspected neurogenetic conditions.**
- **The shortage of medical genetics specialists makes it necessary for neurologists to be familiar with basic concepts in medical genetics that will enable handling some cases and referring when appropriate.**
- **Before and after genetic testing, it is imperative to conduct appropriate genetic counseling. It is ideal to have a specialized genetic counselor provide this service, but in many limited-resource settings, this responsibility lies with the neurologist.**
- **Genetic counseling for massive gene sequencing (such as whole-exome sequencing) should include the expected and unexpected outcomes of testing, the likelihood and type of incidental findings, and which results will or will not be disclosed.**

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KEY POINTS

- **Knowledge about the relationship between genotype (what the DNA looks like) and phenotype (what the patient looks like) has changed dramatically in the past decade.**
- **Mutations in various different genes can cause very similar phenotypes. This is called *genetic heterogeneity*.**
- **Most genes are now believed to be associated with varied phenotypes (phenotypic pleiotropy).**

ongoing treatment trials. As genetic disorders are rare, management is often based on anecdotal reports or small case series. National and international registries are trying to fill this gap, making management guidelines and clinical trials in rare genetic conditions a reality. In selected instances, already available medical therapy might be guided by genotype. In epilepsy, for example, the genotype might suggest that one strategy or medication could be more effective in controlling seizures (eg, the efficacy of the ketogenic diet in glucose transporter 1 [GLUT1] deficiency[1] or avoidance of carbamazepine in Dravet syndrome).[2] Neurotransmitter diseases manifesting with dystonia or parkinsonism might respond differently to different drugs: patients with mutations in guanosine triphosphate cyclohydrolase 1 (*GCH1*) tend to respond to levodopa, while for those with mutations on aromatic L-amino acid decarboxylase (*AADC*), levodopa can be detrimental.[3] More impressively, while the various attempts at genetic therapy for Duchenne muscular dystrophy have shown limited clinical benefit thus far,[4] antisense oligonucleotide therapy is now being implemented for spinal muscular atrophy with dramatic and promising results, with the possibility that it may be further extended to other neurologic conditions.[5]

Despite the complexity of genetic tests and the practical limitations of having them performed, gene testing is currently part of standard care for many neurologic conditions that are discussed later in this article, and it is necessary for neurologists to push through the existing barriers and try to provide the best medical care for patients affected by these conditions.

BASIC CONCEPTS OF GENOTYPE-PHENOTYPE CORRELATIONS

Over the past decades, a wealth of information has been obtained that associates specific genotypes with neurologic disease. With these data, our understanding of genotype-phenotype correlations has also matured. The range of genotype-phenotype correlations is summarized in **FIGURE 1-1**. Down syndrome, caused by trisomy of chromosome 21, was first reported in 1959[6] and is an excellent example of a disorder that often exhibits classic genotype-phenotype concordance (**FIGURE 1-1A**). However, other disorders recognized as hereditary or familial did not always behave so properly. Before gene sequencing was widespread, genetic diseases were classified by linkage to certain loci, or general regions of chromosomes. To search for loci, researchers identified large families in which members had striking and easily identifiable phenotypes. In these relatively simple scenarios, the expectation emerged that all genetic disorders would exhibit classic genotype-phenotype concordance. For some conditions, different loci could be mapped in different families, and it became clear that the genetic basis for these phenotypes could be heterogeneous; therefore, these conditions are said to have *genetic heterogeneity* (**FIGURE 1-1B**). The loci for phenotypes with genetic heterogeneity were often organized by a letter code for the phenotype and then numbered in order of discovery. For example, the first locus described for familial dystonia became DYT1, followed by DYT2, DYT3, and so forth; likewise, for parkinsonism, a series of PARK loci were created (eg, PARK1, PARK2). As linkage was not based directly upon actual genomic features, some loci were not confirmed, resulting in discontinuity and confusion in this organization system and difficulties for the clinicians dealing with it.[7] With more specific identification of genetic causation in larger numbers of patients, most of the vocabulary of the linkage era has been mercifully replaced with language that is based on genomic nucleotide sequence and associated features. The ability to rapidly sequence large

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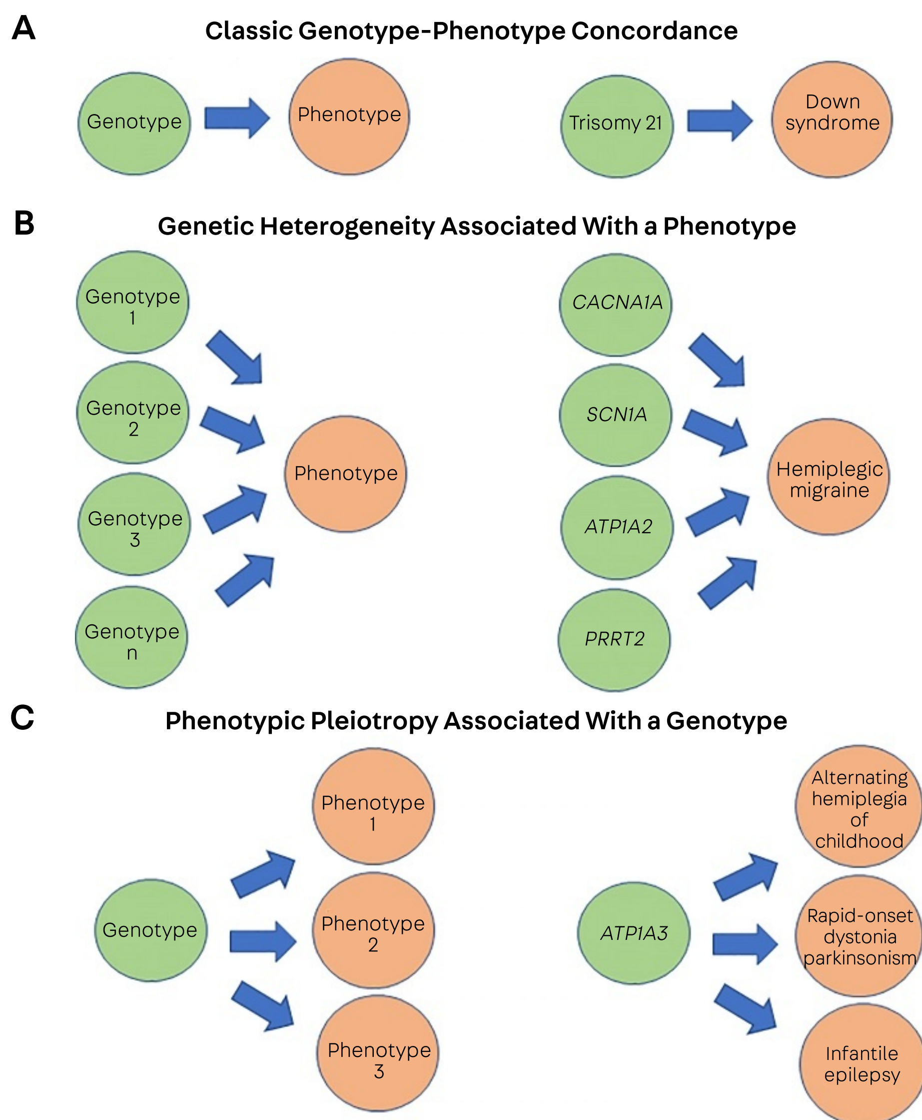

FIGURE 1-1
Genotype-phenotype correlations. *A*, Classic genotype-phenotype concordance. *B*, Genetic heterogeneity, when the same or similar phenotype can be caused by mutations in different genes. *C*, Phenotypic pleiotropy, when mutations in one single gene can cause different phenotypes.

numbers of genes in large numbers of patients has allowed phenotypes that at first seem to be unrelated to be explained by mutations within the same gene. For example, at least three well-characterized disparate phenotypes have been associated with sequence variations in *ATP1A3*: alternating hemiplegia of childhood, rapid-onset dystonia parkinsonism, and severe infantile epilepsy. Another example is sequence variations in *TUBB4A*, causing hypomyelination with atrophy of the basal ganglia and cerebellum or the completely different phenotype whispering dysphonia (also known as DYT4). These observations have become all too common and are termed *phenotypic pleiotropy* (FIGURE 1-1C).

As clinical neurogenetics progresses and more intermediate phenotypes are described, a notion is growing that most neurogenetic conditions present with

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KEY POINT

- **Genetic variability may be normal, predispose the patient to medical conditions (risk variants), cause medical conditions (pathogenic variants), or need further studies to clarify its nature (variant of unknown significance). Interpretation of variants of unknown significance requires the opinion of a medical genetics specialist.**

a phenotypic spectrum, including classic phenotypes, pleiotropic distinct phenotypes with marked clinical characteristics, and more intermediate phenotypes that would not fit into the aforementioned types of presentation.

UNDERSTANDING AND TESTING FOR GENETIC VARIABILITY

The human genome has a range of variation, roughly moving from variations large enough to be seen on a karyotype to smaller ones consisting of a single nucleotide or methylation change. Categories of variation and the appropriate technologies to identify them are summarized in TABLE 1-1, FIGURE 1-2, and FIGURE 1-3.

A correlation exists between pathogenicity and the size of genomic variations and population frequency: large and rare variations are more likely to be pathogenic.[8] Genomic length is measured in base pairs, one unit consisting of two

TABLE 1-1 Categories of Genomic Variations and the Best Testing Technology for Their Detection[a]

Genomic Variation	What It Is	Best Test	Also Detected By
Chromosomal aneuploidy	A chromosomal structural abnormality, ie trisomy or monosomy	Karyotype	Chromosomal microarray
Balanced chromosomal translocation	A chromosomal structural rearrangement in which no loss or gain of genetic material occurs	Karyotype	NA
Unbalanced chromosomal translocation	A chromosomal structural rearrangement in which loss or gain of genetic material occurs	Chromosomal microarray	Karyotype
Chromosomal copy number variation	A region of a chromosome where genetic material is duplicated or deleted	Chromosomal microarray	Karyotype (if >5 Mb)
Chromosomal methylation	Addition of methyl group to cytosine; the mechanism of imprinting	Methylation-sensitive analysis	NA
Gene and exon-level deletions and duplication	Deletion or duplication of entire gene or of specific exons within a gene	Multiplex ligation-dependent probe amplification	Fluorescence in situ hybridization (in some cases)
Short tandem repeat	Run of repeated DNA sequence motifs	Southern blot	NA
Single-nucleotide variant	A single change in the nucleotide sequence	Sequencing	NA
Indel	Insertion or deletion of one or more nucleotides into the DNA sequence	Sequencing	NA

DNA = deoxyribonucleic acid; Mb = megabase; NA = not applicable.

[a] Many (but not all) genomic variations can also be detected by other technologies. Please see article text for discussion of why one modality might be preferred over another.

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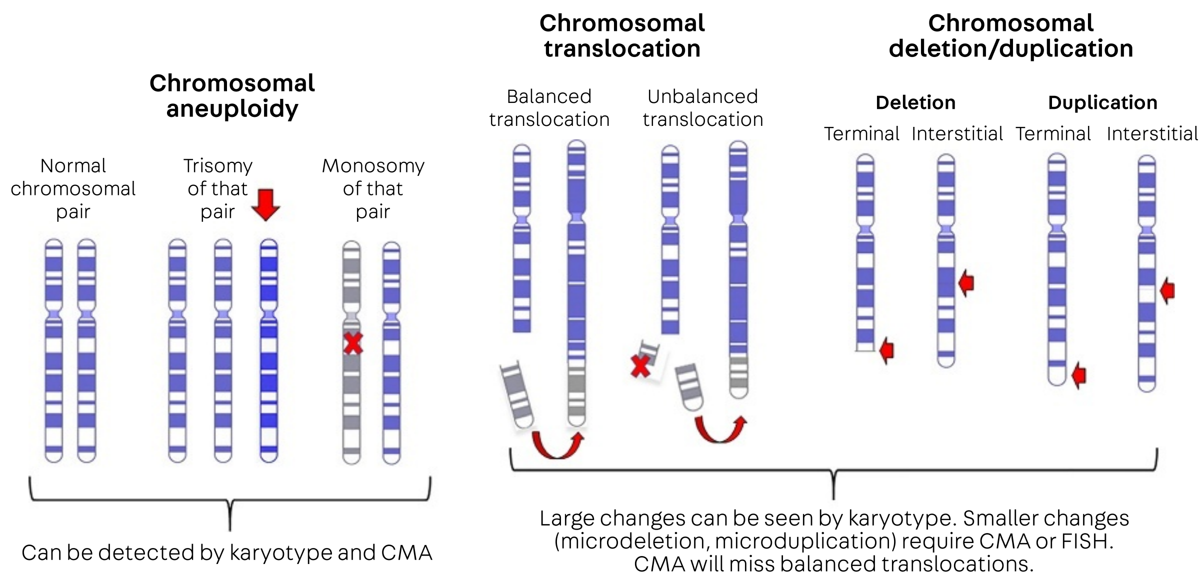

FIGURE 1-2

Genetic variability at the chromosome level. Summary of the main types of abnormalities at the chromosome level and how likely they are to be identified by karyotype, chromosomal microarray (CMA), and fluorescence in situ hybridization (FISH). Deletion or duplication of an entire chromosome is called *chromosomal aneuploidy* and can be detected using traditional karyotype or chromosomal microarray. When parts of one chromosome break off and adhere to another chromosome, the event is termed *translocation*, which is balanced when it does not involve a loss of genomic material or unbalanced when otherwise. Unbalanced translocations can be identified by karyotype or chromosomal microarray, but balanced translocations will be missed by chromosomal microarray. Loss or duplication of genomic information can happen at the end of a chromosome (terminal or subtelomeric deletion/duplication) or from the middle of a chromosome (interstitial deletion/duplication). Karyotype can only visualize large changes (greater than 5,000,000 base pairs), while chromosomal microarray has higher resolution (greater than 100,000 base pairs). FISH is still often used to confirm copy number events found on chromosomal microarray.

complementary nucleotides in the opposing DNA strands. Deletions (or duplications) of megabase pairs (Mb), for example, are more likely to be pathogenic than deletions of a few kilobase pairs (kb). Some regions of the chromosome will be duplicated or deleted in a large proportion of normal subjects; therefore, even a very large event is assumed to be nonpathogenic if very common. The largest chromosome-level events are deletion or duplication of an entire chromosome, resulting in *monosomy* (if a deletion) or *trisomy* (if a duplication). This is called *chromosomal aneuploidy* and can be detected using traditional karyotype. But since chromosomal aneuploidy changes the copy number of an entire chromosome, this event can also be detected via chromosomal microarray, a technique that detects the dose of genetic material with high resolution (greater than 100 kb) and would also identify larger events. Sometimes parts of one chromosome break off and adhere to another chromosome, an event termed *translocation*. When this event does not involve a loss of genomic material, the term *balanced chromosomal translocation* is used. Balanced chromosomal translocation can be detected by karyotype because the translocation changes the chromosome visually, but since no overall change occurs in the copy number of genomic material and only its location changes, chromosomal microarray is unable to detect balanced chromosomal

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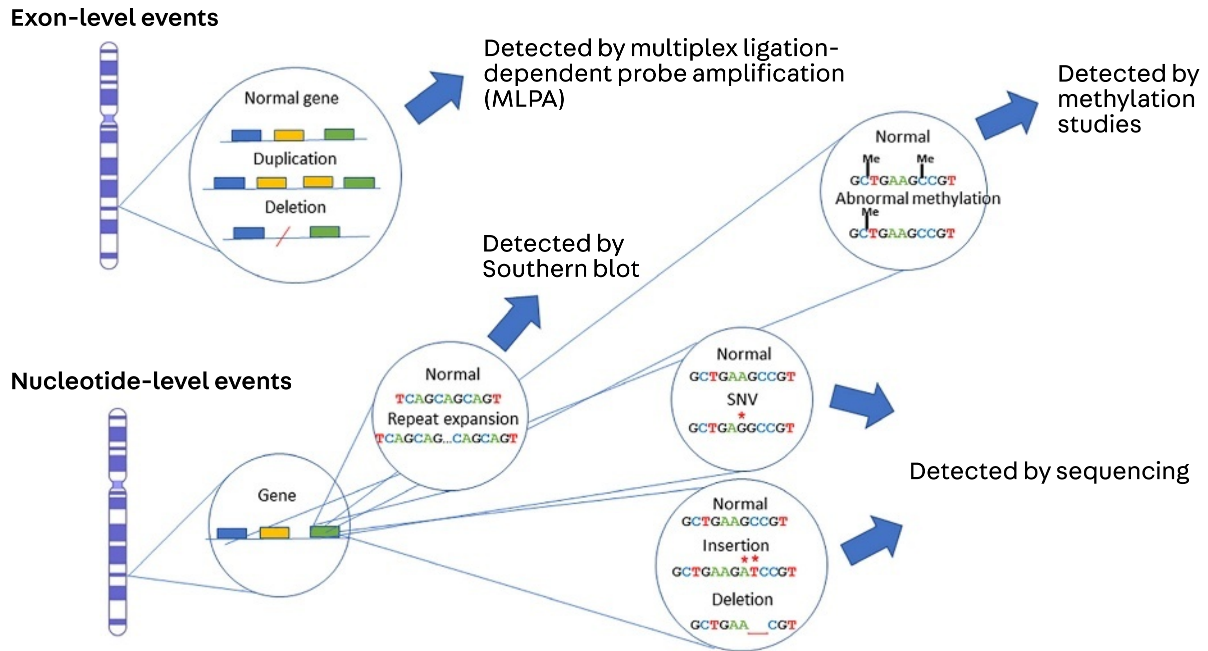

FIGURE 1-3
Genetic variability at the gene level. Summary of the main types of abnormalities at the gene level and the available genetic tests more likely to identify them. Genes are formed by sequences of coding regions (exons, represented as thick colored parts) or noncoding regions (introns, thin lines connecting the exons). An exon can be duplicated or deleted, causing an abnormal protein to be produced, which is often symptomatic. These events are not detected by sequencing and require multiplex ligation-dependent probe amplification (MLPA) for detection. Within an exon, nucleotide-level events can occur, one being an increased number of nucleotides in the form of an amplification of the number of repeats in a naturally occurring short tandem repeat sequence. These events are not reliably detected by sequencing techniques and require DNA Southern blot for diagnosis. When a single nucleotide is changed (single-nucleotide variant [SNV]) or a deletion or duplication occurs within the exon (indels), the change can be detected by sequencing techniques such as Sanger sequencing or massive parallel sequencing (also called next-generation sequencing). Less commonly, the change underlying the phenotype may be abnormal methylation, in which cytosine methylation inherited from one of the parents is abnormal, and the diagnosis will require methylation studies.

translocations. This is an important point; balanced chromosomal translocation is the one chromosome-level event for which karyotype remains superior to chromosomal microarray in diagnostic performance. Balanced translocations may be clinically relevant if the chromosomal breakage site is in the middle of a gene and disrupts function of that gene. If genomic material is lost during a translocation event, the term *unbalanced chromosomal translocation* is used. Unbalanced translocations may be detected both on karyotype and on chromosomal microarray.

Genomic information can be lost in nontranslocation events as well. If a part of a chromosome breaks off and is lost at the end of a chromosome, it is a referred to as a *terminal* (or *subtelomeric*) *deletion*. Alternatively, genomic material can be lost from the middle of a chromosome, which is referred to as an *interstitial deletion*. When genomic material is duplicated at the end of a chromosome or duplicated in the middle of a chromosome, the terms *terminal* (or *subtelomeric*) *duplication* and

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interstitial duplication are used, respectively. Both deletions and duplications are part of normal chromosomal biology and are one type of "copy number events," which are changes in copy number of genomic material, whether that material is coding or noncoding, or large or small. Larger copy number events are more likely to be clinically relevant. Importantly, karyotype can only visualize deletions and duplications larger than 5 Mb. Chromosomal microarray is required to identify copy number variations below 5 Mb down to a resolution of around 100 kb, below which most clinical laboratories do not call copy number variations as these are inevitably not likely to be clinically relevant and are usually benign variations inherited from unaffected parents. An older technology, fluorescence in situ hybridization (FISH) analysis, is an outgrowth of karyotype technology and is still often used to confirm copy number events found on chromosomal microarray.

Genetic events at the exon level are too small to be detected by karyotype or chromosomal microarray and require a different molecular approach. Genes are constructed of exons (coding regions) and introns (noncoding), and disease can result from copy number events at the exon level. Gain of an extra exon is termed an *exon-level duplication,* and loss of an exon is termed an *exon-level deletion*. In general, these duplications and deletions require multiplex ligation-dependent probe amplification (MLPA) for detection, although, in some cases, FISH probes may serve the same purpose.

In any healthy subject, the human genome contains many thousands of repeat regions made up of units of three, four, or more nucleotide repeats, which are called *short tandem repeats*. Some of these regions are in exons (regions within a gene that are transcribed into mRNA, which is subsequently translated into protein amino acid sequence), some are in introns (regions within a gene that are transcribed into mRNA but are then "spliced out" and not translated into protein sequence), some are in untranslated regions of genes (regions at the beginning and end of a gene that are not translated), and many are in intergenic noncoding regions (regions between known genes, which are not transcribed or translated). When the cell divides, the number of repeats can increase (expansion) or decrease (contraction) in these short tandem repeats. Many neurologic disorders, including fragile X syndrome, many spinocerebellar ataxias, myotonic dystrophies, and Huntington disease, are caused by such repeat expansions. These repeat expansions are usually too small to be detected by karyotype, chromosomal microarray, or even FISH probes. For technical reasons, they are not reliably identified using sequencing technologies. Therefore, the best test for identifying repeat expansions remains the DNA Southern blot.

An additional mechanism of genomic variation is methylation. The methylation of cytosines usually silences the region of DNA where it occurs, and the pattern of methylation is inherited in a maternal or paternal pattern depending on whether the chromosome in question comes from the mother or father. This mediates the curious phenomenon of imprinting, whereby the expression of a given phenotype is based upon the parental origin of a chromosomal methylation pattern. Patients have Angelman syndrome if the maternal methylation pattern on chromosome 15q11q13 is missing[9] or Prader-Willi syndrome if the paternal methylation pattern on chromosome 15q11q13 is missing.[10] Testing for methylation requires methylation analysis, which is the test of choice in diagnosing these syndromes as well as other rarer conditions characterized by imprinting.

Nucleotides can be inserted and deleted within exons in a nonrepeat manner as well. This can occur as single-nucleotide insertions or as an insertion of two,

KEY POINTS

- **Multiplex ligation-dependent probe amplification detects dose changes within a gene, chromosomal microarray detects dose changes affecting a small part of the chromosome (greater than 100,000 base pairs), while karyotyping only detects large changes (greater than 5,000,000 base pairs).**

- **Dosage abnormalities can affect only a single gene or a whole region of the chromosome or be in the form of an increased number of repeats in a given sequence that normally is repeated up to a certain number.**

- **Southern blot detects an increased number of repeats in a particular sequence. It is specific for the sequence examined, so if more than one gene with repeats can cause the phenotype observed, a panel of Southern blot for various genes can be requested (eg, testing for various spinocerebellar ataxias at the same time).**

- **In addition to gene sequence and gene dose, other factors affect the production of proteins (epigenetic factors). An important factor affecting gene expression as a disease mechanism is methylation of DNA.**

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KEY POINTS

- **The majority of neurogenetic conditions described thus far are caused by sequence abnormalities that can be detected by DNA sequencing.**

- **Genomic data, including the sequence and dose of genes, needs to be analyzed and interpreted to yield any meaningful clinical result. Interpretation of these results requires the expertise of bioinformaticians and geneticists.**

three, or even more nucleotides. These insertions and deletions are collectively termed *indels* and are a very frequent event in the genome. When the number of nucleotides inserted or deleted is not a multiple of three, it results in a change in the way the ribosomes read the RNA derived from this DNA and is called a *frameshift mutation:* an erroneous sequence of amino acids coded by the DNA downstream of the offending indel. If the indel is a multiple of three, a *nonframeshift* event occurs, and only the amino acids coded by the indel are affected; this may be tolerated or not depending on the specific biology of the protein produced by the affected gene. Indels of either type are generally detected well by traditional Sanger sequencing, provided the insertion is smaller than 700 bp to 1000 bp, because larger insertions may encumber the polymerase chain reaction (PCR) phase of the technique. Indels smaller than 100 bp are, in general, also detected by massively parallel sequencing technologies (so called next-generation sequencing) when combined with the more recent versions of variant detection software.

When one single nucleotide is replaced in the DNA sequence, this event is termed a *single-nucleotide variant*, which may be benign, pathogenic, or of uncertain significance. When it is demonstrated scientifically that a single-nucleotide variant is benign (ie, not associated with any disease and usually present in a percentage of healthy individuals), the term *single-nucleotide polymorphism* is appropriate. When it is demonstrated scientifically that a single-nucleotide variant is pathogenic (ie, associated with a disease and usually absent from healthy individuals) the term *mutation* has been employed, although the more currently preferred term is now *pathogenic sequence variation.* Finally, there exist single-nucleotide variants that are suspect, which have not yet been demonstrated scientifically to be benign or pathogenic. These single-nucleotide variants have been given the label *variant of uncertain significance* until, with scientific progress, they are classified as either benign or pathogenic.

Single-nucleotide variants that occur within the coding region of genes may alter the amino acid codon produced by that sequence of DNA or have no effect whatsoever. Single-nucleotide variants that alter the amino acid are termed *nonsynonymous variants* (also known as *missense variants*), while those that do not result in amino acid change are termed *synonymous variants*. Sometimes a codon is altered so that a premature stop is introduced. This is termed a *nonsense variant* and usually results in an mRNA molecule that is degraded via a process called *nonsense-mediated decay*, which results in no protein product or, in some cases, a truncated protein. Other single-nucleotide variants occur within exon-intron boundaries and, while they do not produce a change in amino acid sequence, can disrupt splicing and result in abnormal inclusion or exclusion of an entire exon. Single-nucleotide variants can also occur within certain untranslated regions of a gene but alter gene translation. Finally, many single-nucleotide variants occur in the noncoding intergenic regions of the genome. Analysis of these single-nucleotide variants is one of the remaining frontiers of genomics. In theory, single-nucleotide variants (and indels) that alter highly conserved regulatory noncoding regions may alter expression of specific genes or cause other genomic alterations. However, this biology is unproven in most cases, and these regions are not sequenced clinically and are only available through genome sequencing.

Single-nucleotide variants are an ideal class of variation to be detected by sequencing. Sanger sequencing techniques, in which one gene at a time is generally amplified and then sequenced, has been largely displaced by massively parallel sequencing technology. Using massively parallel sequencing technology, total

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genomic DNA is fragmented randomly into roughly 100-bp pieces and then subjected to a library capture in which molecular baits (short complementary sequences) pull down the genomic regions of interest for sequencing. In the case of exome sequencing, which targets only coding regions, the library capture contains baits for the exons of roughly all 20,000 known genes. Noncoding regions are therefore not sequenced. Once the pieces of DNA are pulled, they are sequenced by a huge number of sequencers simultaneously; although this technique is referred to as *massively parallel sequencing*, the term *next-generation sequencing* is also still used.

The many small pieces of sequence produced are like a puzzle that must then be assembled and mapped to the known sequence of the human genome, with variations identified and statistical corrections applied. Next, the results must be annotated so that the end product is a list of all variations in each gene, where the variant is located, whether it is synonymous or nonsynonymous, whether it is common or rare, and even whether the variant is predicted to be deleterious or benign. This analysis requires high-performance computing, and interpretation requires the expertise of bioinformaticians and geneticists.

TABLE 1-2 summarizes the main sequencing techniques currently used and their strengths and weaknesses. It is important to understand that massively parallel sequencing does not detect most classes of genomic variation. In general, all genomic variation in which the sequence of DNA is not altered will be invisible to any sequencing technology. Therefore, chromosomal aneuploidy, translocations, and chromosomal copy number variants are not detected by current sequencing technology. Likewise, exon-level deletions and duplications are not visible by sequencing, since they change the copy number of an exon, not the nucleotide

TABLE 1-2

Practical Aspects of Common Commercially Available Gene Sequencing Testing Methods

Test	Main Strengths	Main Weaknesses
Single-gene sequencing	Cheap, quick, and easy to interpret; as it usually targets a known disease gene, less likely to return unexpected findings	Not useful for cases with atypical phenotype; not practical for phenotypes with genetic heterogeneity
Gene panel sequencing (massively parallel methods)	When multiple genes can cause similar phenotypes, panel testing is usually cheaper than testing for each gene sequentially	Coverage and cost of gene panels vary significantly, and decision to request might require in-depth knowledge of genotype-phenotype correlations for that condition
Exome sequencing (massively parallel methods)	Assays approximately 20,000 genes simultaneously, making this test most efficient for disorders with genetic heterogeneity or for atypical phenotypes	Targeting of exome analysis by the testing laboratory is performed according to clinical information provided, so thorough phenotyping and appropriate interaction with medical genetics is necessary
Genome sequencing (massively parallel methods)	Extends coverage for noncoding regions	Currently reserved for research settings

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sequence. Instead, the remaining (normal) allele will be sequenced, providing a false-negative sequencing result. Repeat expansions are generally not detectable by massively parallel sequencing if the repeat expansion is greater than 100 bp. This is because the newer sequencing technologies work off 100-bp fragments that must then be mapped back after sequencing to the reference human genome. If the repeat region is greater than 100 bp, the fragment containing only repeat sequencing products cannot be mapped to a unique location in the genome. Therefore, even with the recent advances in sequencing technology, diagnostic tests such as karyotype, chromosomal microarray, FISH, MLPA, and Southern blot will remain valuable techniques for the foreseeable future.

Given the rapidity with which new genetic causes of neurologic conditions are being discovered, exome sequencing is probably the most cost-effective approach to diagnosis in phenotypes with great genetic heterogeneity, because the coverage is extensive and data can be reanalyzed at a later date as more genes are discovered and the significance of variants of unknown significance is clarified. However, one weakness of exome sequencing is the inability to detect exon-level deletions and duplications. Deletion/duplication testing is sometimes offered as part of more focused massively parallel panels, but one may need to determine if a particular laboratory offers this technique. To optimize cost-effectiveness, it is important to consider that genetic causes of more early and dramatic phenotypes (such as global developmental delay and intellectual disability, epileptic encephalopathy, and infantile neurodegeneration) are often de novo in inheritance pattern; therefore, it is relevant to know if parental DNA could be tested to clarify variants of unknown significance that are difficult to interpret.

NEUROGENETICS IN THE NEUROLOGIC SUBSPECIALTIES

Entire subfields now exist within neurology that barely existed 2 decades ago, such as genetics of epilepsy, genetics of movement disorders, and genetics of neuromuscular diseases. A detailed discussion of every genetic cause of every neurologic presentation is beyond the scope of this article. Instead, very broad general principles are described that may facilitate organization of the initial approach to eight key clinical presentations, with the understanding that many nuances exist within each category of presentation and that workups are best performed in consultation with domain-specific experts (subspecialists with discrete training, expertise, and experience with a particular group of conditions). These notions apply when considering genetic etiologies; therefore, nongenetic causes should always be excluded when appropriate.

Global Developmental Delay and Intellectual Disability

Global developmental delay is one of the most common reasons for an outpatient child neurology office visit, and it is defined as delay in at least two of the four realms of child development (gross motor, fine motor, speech and language, and social development). If the patient is older than 5 years of age, has impairment in adaptive functioning, and has a full score of less than 70 on an IQ test, the term intellectual disability is appropriate. Several classic genetic syndromes have this presentation, including fragile X syndrome, the most common cause of intellectual disability in boys. A myriad of other syndromes likewise have this presentation, and, in younger patients, other sentinel signs/symptoms may not yet be present. Therefore, a broad screening approach has been recommended by professional societies, including a survey of genomic architecture with a

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chromosomal microarray, ideally combined with karyotype. Fragile X syndrome is caused by triple repeat expansion in the *FMR1* gene and is not detected by chromosomal microarray. Therefore, specific Southern blot testing for this disorder is usually performed early in the workup.

If these tests are normal, varied options for further diagnostic testing are available. Global developmental delay and intellectual disability are prime examples of phenotypes with extreme genetic heterogeneity, as literally thousands of diagnostic possibilities exist with little to differentiate them clinically. Therefore, a massively parallel sequencing strategy (such as neurodevelopmental gene panels and exome sequencing) is the best next test to offer. Other nongenomic diagnostic tests that may help narrow the field include specific neurometabolic enzyme testing and *N*-glycosylation and *O*-glycosylation screening for congenital disorders of glycosylation. Finally, if Angelman syndrome or Prader-Willi syndrome are at all suspected, separate methylation-sensitive PCR should be ordered, as this is the best first test to detect the various causes of those syndromes. A normal methylation pattern of chromosome 15q11.2-q13 rules out Prader-Willi syndrome, and, if clinical suspicion for Angelman syndrome is strong, sequence analysis of *UBE3A* should be pursued next. Sequential testing with chromosomal microarray/reflex karyotype, fragile X, and exome sequencing can approach a diagnostic yield of up to 60%.[11]

Autism

Autism spectrum disorder is defined by persistent deficits in social communication and social interaction and restricted or repetitive patterns of behavior, interests, or activities, usually present in the early developmental period, causing significant impairment, and not better explained by intellectual disability.[12] Despite early evidence for a genetic basis for some forms of autism, as seen in fragile X syndrome, further advances in testing technologies were required before the breadth of genetic causes of autism spectrum disorder became apparent. This means that the diagnostic approach to autism spectrum disorder is similar to that for global developmental delay/intellectual disability: chromosomal microarray with reflex karyotype (ie, an immediate karyotype if the chromosomal microarray is normal) and fragile X testing are indicated as first-line tests. As with intellectual disability, most genetic causes of autism spectrum disorder are de novo in inheritance[13] (with obvious exceptions, eg, fragile X syndrome). If the architecture of the genome has been verified as normal, then massively parallel sequencing strategies are indicated, much like in neurodevelopmental disorders, with the same caveat regarding detection of exon-level deletions and duplications, considering neurometabolic causes, screening for congenital disorders of glycosylation, and specific methylation testing for Angelman syndrome. The American College of Medical Genetics has published guidelines for the evaluation of individuals with developmental delay.[14]

Epilepsy

Epilepsy is perhaps the most extreme example of genetic heterogeneity confronting the diagnostician. At present, hundreds of genes are associated with epilepsy causation, and indications are that thousands may ultimately be discovered as research advances. However, many of the same principles that underlie the genetic pathogenesis of global developmental delay/intellectual disability and autism spectrum disorder also hold true for epilepsy. While a number of well-described X-linked and autosomal dominant epilepsy disorders

KEY POINTS

- Most pathogenic genetic variability is caused by variation of sequence or dose of the DNA. The techniques used to detect these types of abnormalities are very different, so ordering the correct test is as important as targeting the right gene.
- Genomic data can be stored indefinitely and reanalyzed as the knowledge about genomics evolves.
- Given the rapidity with which new genetic causes of neurologic conditions are being discovered, exome sequencing is probably the most cost-effective approach to diagnosis in phenotypes with great genetic heterogeneity without a high chance of chromosomal events, but many exceptions exist.
- Global developmental delay, intellectual disability, autism spectrum disorder, and epileptic encephalopathies are frequently caused by chromosomal-level events, so chromosomal microarray and karyotype are likely to be helpful.
- Autism, intellectual disability, and epileptic encephalopathy are all conditions with great genetic heterogeneity, and each can be caused by mutations in more than 50 known genes.

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are known, most causes described (particularly the early-onset epileptic encephalopathies) are of de novo inheritance. As previously mentioned, the best first test for molecular diagnosis, independent of the electroclinical epilepsy syndrome identified, is chromosomal microarray, with karyotype performed if the microarray is normal. Should chromosomal microarray and karyotype be normal, some form of massively parallel sequencing approach (with the aforementioned caveats) would be the most appropriate next step.

Neurodegeneration

Neurodegeneration is a broad category of presentation that can range from young children with catastrophic loss of developmental milestones and deterioration of neurologic function to children who initially present with global developmental delay but over time take on a more ominous clinical course to older patients who may develop dementing symptoms, ataxia, movement disorders, or any combination of progressive neurologic signs. Rather than discuss each of the myriad causes separately, a general approach to genetic diagnostics in these scenarios is presented here. First, in the event of presentation of classic signs of a specific disorder to a keen diagnostician (eg, retinal cherry red spot, increased startle response, and loss of motor skills in an infant is highly suggestive of Tay-Sachs disease; hypotonia, generalized muscle weakness, respiratory distress, and cardiomyopathy in an infant is suggestive of Pompe disease; onset of dystonia, hepatitis, psychiatric symptoms, and a Kayser-Fleischer ring in a young adult are almost pathognomonic of Wilson disease), the pursuit of specific enzyme or metabolite testing for the suspected neurometabolic disorder is the best approach. In other cases, the presentation (such as chorea and declining cognitive performance in a young adult with an autosomal dominant family history of the same) may suggest a single gene for immediate testing (in this case Huntington disease, which is diagnosed by Southern blot testing of the trinucleotide repeat on the *HTT* gene). Screening metabolic testing for peroxisomal (very-long-chain fatty acid testing), lysosomal (lymphocyte lysosomal enzyme panel testing), and mitochondrial (serum or CSF lactate, pyruvate, plasma and CSF amino acids) disorders as well as other tests, such as urine organic acids, acylcarnitine profile, skeletal survey, and CSF neurotransmitters, can be used to detect abnormalities in specific systems. For more information, refer to the article "Testing for Inborn Errors of Metabolism" by Jennifer M. Kwon, MD, MPH, FAAN,[15] in this issue of *Continuum*.

Findings from these preliminary forays can then be used to guide subsequent genetic testing. In many cases, however, the pattern of neurodegeneration is so nonspecific that no single class of diagnosis is suggested, or it may be associated with such genetic heterogeneity that a broader approach is indicated. Some cases may initially present as global developmental delay/intellectual disability, autism spectrum disorder, or epilepsy until their true neurodegenerative etiology becomes apparent. As a general rule, autosomal recessive single-gene disorders and mitochondrial genomic disorders are enriched in this category of presentation, altering our approach to diagnostics. The more traditional de novo copy number deletion syndromes are less likely to have a neurodegenerative course. For this reason, chromosomal microarray may not be the first test indicated for neurodegeneration. If preliminary metabolic screening has indicated a particular set of genes, the genes can often be tested by means of a gene "panel," with due attention also paid to the possibility of exon-level

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deletions/duplications. If preliminary metabolic screening suggests a disorder of mitochondrial function, these disorders can be encoded on the mitochondrial genome as well as through a number of nuclear genes (mostly autosomal recessive inheritance). Therefore, genetic testing in these cases may involve mitochondrial genome sequencing (which often must be ordered separately from other types of sequencing) and massively parallel sequencing that involves a so-called mitochondrial panel on the autosomal genome. Neurodegeneration presenting as ataxia (particularly the spinocerebellar ataxias) may be due to repeat expansions, which are not detected by next-generation sequencing. Specific Southern blot testing for these disorders would be necessary. If preliminary metabolic screening does not detect a class of disorder of peroxisomal, lysosomal, or mitochondrial function, a broader approach through exome sequencing may be indicated. If these tests are unsuccessful, chromosomal microarray (with follow-up karyotype if the microarray is normal) can be offered when neurodegeneration may be due to an unusual presentation of a disorder of chromosomal copy number.

KEY POINT

- In neurodegeneration, movement disorders, and neuromuscular disorders, excluding compatible metabolic causes is paramount. Often, the opinion of a subspecialist is necessary before genetic testing.

Microcephaly

Occipital-frontal head circumference two standard deviations or more below the mean can be categorized as prenatal (present at birth) or postnatal in onset, with markedly different genetic evaluations for each. Some forms of microcephaly are also associated with specific brain malformation patterns, which are discussed in the next section. Prenatal microcephaly is associated with mutations in a number of genes with autosomal recessive inheritance,[16] and these can be sequenced in a massively parallel approach through a panel or exome sequencing (taking caveats into consideration) followed by chromosomal microarray. Recessive disorders can feature an inherited pathogenic sequence variation in a gene on one allele combined with a deletion of the same gene on the other allele.

Postnatal microcephaly presents with normal head size at birth but with progressive deceleration in head growth, usually through infancy and toddlerhood. The genetic landscape of postnatal microcephaly disorders is markedly different than that of prenatal microcephaly; for this reason, a clear charting of head size over time since birth is the essential first step of the evaluation. In general, postnatal microcephaly disorders overlap with the previously discussed disorders presenting with global developmental delay/intellectual disability, epilepsy, and even autism spectrum disorder, and a similar approach could be followed except for fragile X syndrome, which is not a common cause of microcephaly.[17]

Brain Malformations

Over the past decades, detailed knowledge has emerged regarding the genetic causes and classifications of structural brain malformations.[18,19] These include specific patterns of premigrational, migrational, and postmigrational forebrain malformations, hindbrain malformations, and syndromes that involve a combination of these patterns. For more information, refer to the article "Nervous System Malformations" by John Gaitanis, MD, and Tomo Tarui, MD,[20] in this issue of *Continuum*. Thorough interpretation of the findings on MRI by an expert in these disorders is a necessary first step in the diagnosis, because what is needed is not merely the identification of the various findings on the image but also the insight to put findings together into rare but recognizable patterns. One must consider that several categories of brain malformations are usually due

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to prenatal insults, such as viral infections or ischemic events, and that some patterns of brain malformation are so specific as to be associated with a defect in a single gene. Other brain malformations are associated with specific congenital anomalies elsewhere in the body that may suggest a diagnosis. However, most patterns of brain malformation exhibit sufficient genetic heterogeneity that a broader genome-wide approach to testing is indicated. As with global developmental delay/intellectual disability and epilepsy (which are frequent copresenting signs with brain malformations), the best first test is usually chromosomal microarray with reflex karyotype, followed, when needed, by massively parallel sequencing panels for brain malformation. Exceptions to this approach abound, making the opinion of a specialist even more valuable (CASE 1-1).

Neuromuscular Disorders

Hereditary neuropathies may present with motor or sensory peripheral nerve dysfunction or both. Recognition of whether a neuropathy is axonal or demyelinating and determination of a clear inheritance pattern are in the realm of most neurologists, but familiarity with the growing list of genetic conditions associated with neuromuscular disorders may not be. For this reason, consultation with a neuromuscular specialist is advisable in suspected genetic cases in addition to nerve conduction studies and EMG, which are often an essential component of the evaluation before a genetic test is ordered. Except for Charcot-Marie-Tooth disease type 1 (which is largely caused by duplication of *PMP22*) and X-linked axonopathies (mostly ascribed to sequence variations in *GJB1*), other forms of hereditary neuropathies, such as autosomal dominant axonopathies (eg, Charcot-Marie-Tooth disease type 2), can be caused by abnormalities in dozens of different genes. Previous strategies that tested the most common gene in each

CASE 1-1

A 2-year-old boy with a history of hearing loss but no other neurologic symptoms presented with acute head trauma and underwent a CT scan. The image showed no acute changes but demonstrated structural abnormalities, so a follow-up visit was scheduled and an MRI was ordered. The MRI showed agenesis of the corpus callosum, polymicrogyria, and ventricular dilation. He had no history suggestive of prenatal injury and no family history of neurologic abnormalities.

A karyotype was ordered, which disclosed no abnormalities, then a chromosomal microarray was requested and showed two microdeletions of unknown significance. A referral to a medical geneticist with an interest in brain malformations was then requested. The geneticist reported that the MRI findings were highly suggestive of Chudley-McCullough syndrome and ordered single-gene testing for *GPSM2* mutations, which showed compound heterozygous mutations of the gene, providing a definite diagnosis. Since the condition is autosomal recessive, further reproductive counseling was provided for the parents.

COMMENT

This case illustrates how an early opinion of a subspecialist can spare unnecessary tests and allow for early reproductive counseling.

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phenotype first, followed by sequential testing of the other likely culprits, is increasingly giving way to panel-based testing for dominant or recessive and demyelinating or axonal presentations. Some laboratories offer massively parallel all-inclusive hereditary neuropathy panels, but it should be remembered that the most common duplication of *PMP22* requires copy number testing.

Hereditary neuropathy can also be part of several autosomal recessive neurometabolic disorders, such as Refsum disease, metachromatic leukodystrophy, and Krabbe disease, and appropriate testing should be performed if indicated. Other conditions associated with neuropathy include mitochondrial diseases, Friedreich ataxia, X-linked adrenomyeloneuropathy, and Pelizaeus-Merzbacher disease.

Primary disorders of muscle are a large and varied group of conditions, with diverse genetic etiologies. Forms of the same disorder may present at birth, in infancy or early childhood, or later in adult life. With few exceptions, consultation with an experienced specialist is indicated to guide genetic testing. Age of onset, creatine kinase levels, nerve conduction studies and EMG, and muscle biopsy with specific immunohistochemical and biochemical assays may yield a pattern suggestive of a diagnosis. In recognizable cases, such as Duchenne muscular dystrophy, immediate testing of a single gene may be indicated. Testing of the *DMD* gene is a prime example of the importance of exon-level deletions in pathogenesis, as up to 70% of pathogenic mutations in this gene are deletions of one or more exons, and genetic testing approaches should take this into account. Gene panel approaches might be indicated when confronted with a congenital myopathy, congenital muscular dystrophy, and a limb-girdle muscular dystrophy. If a mitochondrial etiology is suspected either by the specifics of the clinical presentation or results from muscle biopsy, mitochondrial genome sequencing may be helpful.

Movement Disorders

Movement disorders, including tremor, dystonia, myoclonus, chorea, and, less often, parkinsonism, often accompany neurodegenerative conditions in children, as discussed earlier in this article. Isolated movement disorders previously characterized as predominant "pure" movement disorders were often labeled *primary* before genomics identified the causes for many of these conditions. In practical terms, a primary or idiopathic movement disorder is suspected when no history of brain injury is present, brain imaging through structural MRI is normal, and laboratory investigations (including testing for inflammatory or metabolic disease when indicated) are negative. That rules out, for example, Wilson disease, systemic lupus erythematosus, and anti–*N*-methyl-D-aspartate (NMDA) receptor autoimmune encephalitis, to name a few. Neurotransmitter diseases can be detected by biochemical abnormalities in the CSF but most often present as primary movement disorders,[21] and, in addition to dopa-responsive dystonia (DYT5), they can present with cerebral palsy–like phenotypes, which also respond to dopaminergic therapy.

Most primary movement disorders are caused by sequence variations or small deletions or duplications within the causative gene, although copy number variants detected through chromosomal microarray have been reported in association with primary movement disorders and remain a possibility.[22] The genetic heterogeneity and phenotypic pleiotropy of many genes causing movement disorders make a case for a broad approach to gene testing, either through a gene panel or exome sequencing. Today, most commercial gene panels

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are targeted to a key phenotype, such as dystonia, juvenile parkinsonism, chorea, paroxysmal dyskinesia, or ataxia. Nevertheless, with the expanded spectrum of many conditions, it is becoming clear that a significant overlap exists between these key phenotypes. Genes causing recessive ataxias, for example, are known to masquerade as primary choreas,[23] and many neurodegenerative conditions with expanded spectrums can present with movement disorders as their initial manifestation for months or years. Therefore, it is imperative that the clinician ordering the tests be familiar with the conditions and their variability as well as with the testing technologies and their limitations. Often, the opinion of a movement disorders specialist or a neurogeneticist is advisable, but in the context of a movement disorder phenotype highly suggestive of a particular condition (eg, Huntington disease), the testing for a single gene may be the initial choice.

Some idiopathic movement disorders with likely genetic risk factors (ie, positive family history) with no clear mendelian inheritance have not been associated with pathogenic sequence variations in any gene despite extensive studies in past decades. These include Tourette syndrome and essential tremor, both of which require no genetic testing for diagnosis and management at the present time.

TRENDS AND DIRECTIONS FOR THE FUTURE

Genetics data are unintelligible for clinicians without proper training. To be interpreted, these data require processing with up-to-date software followed by interpretation by clinical geneticists and sometimes subspecialists, who match symptoms with the data (CASE 1-1). Interpretation continuously matures as more genetic studies are performed, more subjects are tested, and more pathogenic variants are described. These data are not all localized in one place but are spread out over different websites and electronic resources, which require professionals in bioinformatics who are familiar with the analytic environment. Domain specialists who are very familiar with the clinical manifestations of certain conditions and are continuously updated on the neurogenetics involved in them are also an important part of the interpretation process. Many of the exome sequencing tests or chromosomal microarrays that failed to return known pathogenic variants a few years ago may need to be reinterpreted, or at least the variants of unknown significance should be checked, as their significance might have become clear.

Much genetic testing is performed in research settings, either because of cost or because of recruitment of patients with specific phenotypes into genetic studies. An obvious benefit of research sequencing is that domain specialists are often involved and contribute to the interpretation of findings. Research analyses can be repeated as more evidence becomes available or when the patient, under continued follow-up by a research team that includes specialists, presents with new symptoms that can aid the interpretation. Overall, maintaining a network of contacts in the field of neurogenetic research can be an ideal way to obtain valuable clinical advice and sometimes genetic testing on a research basis. Nevertheless, neurologists with busy and often isolated clinical practices with little time or opportunity to cultivate these contacts may strive to grow their network of referrals and use a flexible threshold for referring suspected genetic conditions that they feel more or less comfortable dealing with. While a network of contacts might be a temporary solution, it is imperative that the number of medical geneticists increases to meet the growing demands for referral and genetic testing.

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CONCLUSION

Currently, neurologists dealing with suspected or confirmed neurogenetic conditions often need the opinion of a medical genetics specialist and a genetic counselor. These resources may be unavailable in many regions throughout the world, but with a good network of contacts in the medical genetics field and within neurologic subspecialties, this shortcoming can be mitigated. In some conditions in which genetic etiologies are prominent and targeted testing is the first choice, it may be more practical for the neurologist to order the test and perform pretest and posttest genetic counseling. With the increased complexity of genetic testing and genomic data available, the expansion of the field of neurogenetics is inevitable.

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USEFUL WEBSITES

CLINVAR

ClinVar is an expertly curated public archive of reports of genomic variations and phenotypes, with supporting evidence.
ncbi.nlm.nih.gov/clinvar/

DEVELOPMENTAL BRAIN DISORDERS DATABASE

The Developmental Brain Disorders Database provides a repository of genes, phenotypes, and syndromes specifically targeted at neurodevelopmental disorders curated by domain specialists.
www.dbdb.urmc.rochester.edu/home

ENSEMBL

The Ensembl genome browser is another invaluable tool for visualizing regions of interest and their associated annotations.
ensembl.org/index.html

GENEREVIEWS

GeneReviews provides chapter-length information on clinical scenarios and specific single-gene disorders. It is written by domain experts, with authoritative recommendations regarding counseling, testing, and management.
ncbi.nlm.nih.gov/books/NBK1116/

ONLINE MENDELIAN INHERITANCE IN MAN

Online Mendelian Inheritance in Man (OMIM) is a comprehensive compendium of genes associated with genetic disorders as well as phenotypes inherited in clear familial patterns for which no specific genetic mutation has thus far been described.
ncbi.nlm.nih.gov/omim/

SEQUENCE VARIANT NOMENCLATURE

The Sequence Variant Nomenclature website provides technical information on the description of sequence variants.
varnomen.hgvs.org/

UNIVERSITY OF CALIFORNIA SANTA CRUZ GENOME BROWSER

The University of California Santa Cruz Genome Browser is an invaluable tool for visualizing regions of interest and associated annotations for these regions.
genome.ucsc.edu/

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