Review

Genomic Diagnosis for Neurodevelopmental Disorders: Revolution, Evolution, and Current Reflections on the “Rational Diagnostic Evaluation”

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Review

Genomic Diagnosis for Neurodevelopmental Disorders: Revolution, Evolution, and Current Reflections on the "Rational Diagnostic Evaluation"

Running title: Genomic Diagnosis for NDDs

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Article Summary:

This review presents a perspective on the current rational evaluation of child with NDDs given the repertoire available to the pediatric practitioner facing this challenge

Abstract

Neurodevelopmental disorders (NDDs) are a class of childhood-onset conditions that affect brain development and function. NDDs have a heterogeneous etiology, a wide genetic and clinical variability and generally lead to impaired cognition, communication, psychomotor skills, and adaptive behavior. These disorders include intellectual disability (ID), autism spectrum disorder (ASD), and developmental and epileptic encephalopathies that manifest during childhood. Over the past 2 decades, genetic research has discovered more than 1,500 genes in different signaling pathways that are involved in NDDs, including many transcriptional regulators such as DNA/histone modifiers and chromatin-regulatory protein complexes. These same investigations have led to the accessibility and availability of next-generation sequencing in the assessment of children with NDDs in the clinical setting. The advances have dramatically altered the approach to the genetic diagnostic assessment of the child with NDDs and have increased the diagnostic yield of genetic testing in the pediatric setting. The purpose of this review is to provide the historical background to the rational assessment of child with an NDD and present a perspective on the current evaluation given the modern repertoire available to the pediatric practitioner facing this challenge in the clinical setting.

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One of the most common presentations to the primary care pediatric setting is the individual with a neurodevelopmental disorder (NDD). The patient usually has a global developmental delay (GDD), intellectual disability (ID), and/or autism spectrum disorder (ASD). The question of a genetic cause or syndrome arises because of parents' questions about prognosis and risk for the condition to reoccur in future pregnancies. The recognition of phenotypic signs leads to a referral to genetics or child neuropsychiatry. The reason for the specialist referral is to establish an etiologic diagnosis to inform management. Currently, there exists a lack of clarity among health care professionals regarding the necessity of genetic testing [1]. The purpose of this review is to provide the historical background to the rational assessment of child with NDDs and to present a perspective on the current optimal evaluation given the modern repertoire available to the pediatric practitioner facing this challenge in the clinical setting. As genetic testing options rapidly expand, the clinical community needs to be mindful of their individual strengths and limitations in order to select the most

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appropriate diagnostic pathway. While the term NDDs can often include other neurologic and behavioral conditions, this review will focus on the assessment of children with GDD/ID/ASD.

In *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) the designation GDD is used to explain developmental disability in children–younger than age five [2,3]. About 5%-10% of the pediatric population experiences GDD [2]. ID is a term used for children older than 5 years of age when proper standardized measurements of developmental skills can be performed [4]. It is a variable, heterogeneous manifestation of central nervous system dysfunction with a prevalence of about 3% of the global population [4–6]. According to the DSM-5 [3], ID is defined as a defect in intellectual functioning and adaptive behavior starting before age 22 years, influencing the conceptual, social, and practical domains in daily life. ASD is a complex developmental disorder with early onset, manifesting as deficits in social communication and interaction and the presence of restrictive/repetitive behaviors and interests and/or sensory disorders interfering with daily functioning. Having a child with a NDD has a major impact on families regarding many aspects of daily life, and the financial burden on society is huge [7]. ID/ASD are both clinically and genetically heterogeneous. Clinical heterogeneity (diversity) is reflected by a wide range of different ID/ASD conditions due to variants of a large number of genes and by the broad clinical spectrum caused by mutations in the same gene [8].

ID/ASD are often one component of a broader condition including disorders of known etiology such as Down and fragile X syndromes. Associated neurodevelopmental comorbidities, such as epilepsy, attention deficit hyperactivity disorder (ADHD), are common, and specific characterization of these comorbidities is important in management. We will discuss a phenotypic classification of presentations of ID/ASD below. The genetic heterogeneity of NDDs is reflected by the large number of genes known and estimated to be involved in ID (about 2,000) and ASD (about 1,000), which has led to the latest ID/ASD gene panels used in diagnostic laboratories containing about 1,500 genes [9,10]. A significant genetic overlap between ID, epilepsy, and ASD has also been shown [11,12].

The Importance of Diagnosis

The clinical and genetic heterogeneity complicate the process of obtaining a precise etiological diagnosis, which is of paramount importance for the affected individual, the family, and the care providers. Although the labeling process might seem stigmatizing, diagnosis provides prediction, and it is usually sought by the family members [13], who want to know why and how it happened and if it will happen again. Knowing the etiology can be liberating, providing relief following years of uncertainty. Precise diagnosis may help establish an accurate recurrence risk, predict the prognosis with relative certainty, and organize appropriate laboratory testing, avoiding a diagnostic evaluation of unnecessary expense and invasiveness. The diagnosis and the knowledge of the natural history together make it possible to plan specific management and treatment, and may help the family in coping with the potential serious manifestations and/or developmental disabilities [14,15] (see Figure 1). Referral to support groups, the strong stimulus to initiate a new parent support group, or arrangement of a meeting with other parents and children with the same disorder are examples of how much can be accomplished in the care setting with an established diagnosis [16]. As clinicians we have experience with other individuals having the same disorder, access to management programs, knowledge of the prognosis, awareness of research on the disease, and many other elements that when shared with the parents will give them a sense that some control is possible [17,18].

In addition, continuous scientific advancements and ongoing studies are making feasible the specific treatment of a number of genetic disorders. The treatment is usually directed against the mechanism of the disease and thereby alters its natural history [19–22] (See illustrative examples in the Appendix). In our experience, the communication of diagnosis has been crucial for the therapeutic alliance and is the first step toward the study of behavioral phenotype [25].

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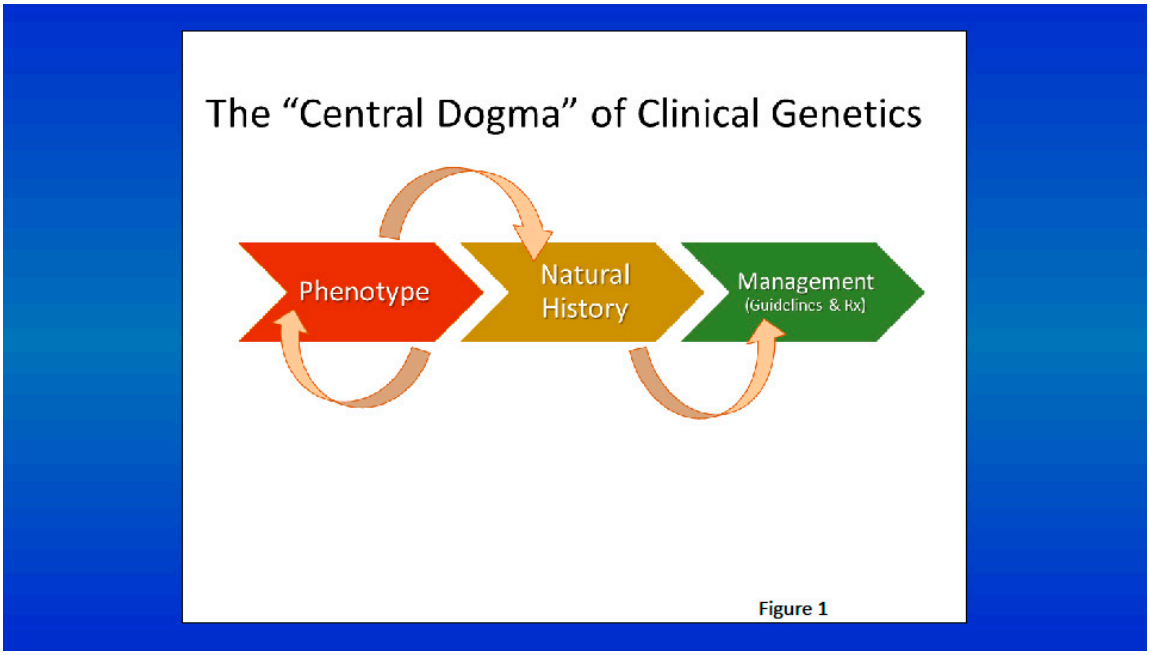

Figure 1. The central dogma of medical genetics, like the central dogma of molecular biology, progresses from the description of the phenotype of a genetic condition to the characterization of its natural history, which is vital to the establishment of management guidelines and treatment (from Cassidy & Allanson's Management of Genetic Syndromes, eds, Carey JC, Battaglia A, Viskochil D, and Cassidy SB. Hoboken, NJ, John Wiley & Sons, 2021).

When GDD/ID/ASD are identified in a child, there is a shared sense of urgency to determine the causative factors. Given the potential impact of such a diagnosis and the hundreds of conditions known to cause GDD/ID/ASD, hypothetically one could perform a number of investigations. Powerful recent advances in technologies to analyze the genome have had a profound impact on the practice of medical genetics, both in the laboratory and in the clinic. Numerous techniques are now available to diagnose a particular phenotype, and while traditional techniques remain efficient tools in certain situations, higher-throughput technologies have become the de facto laboratory tool for diagnosis of most conditions. Maintaining a clear understanding of the rapidly evolving landscape of diagnostic tests, and their limitations, presents a challenge for non-genetics professionals. Therefore, selecting the right technology is challenging, and the wrong choice may lead to prolonged time to diagnosis, high costs, or even a missed diagnosis. Literature review, coupled with our own experience, highlights the need to further educate primary care clinicians in the uses and limitations of genetic testing for NDDs [26].

A rational approach to a diagnostic evaluation promises to provide significant benefits to the individual, family, and practitioner. For reaching a diagnosis, two main approaches are recognized historically: "phenotype-first" and "genotype-first". Phenotype-first refers to the traditional approach to the recognition and delineation of a diagnostic entity. All of the conditions with eponyms exemplify this: a set of patients in the clinical setting are recognized to have overlapping manifestations and are documented, suggesting that the patients represent a diagnostic entity (e.g., Coffin-Siris syndrome). The phenotypic spectrum, the natural history, and more recently the etiology are characterized in later publications. Genotype-first refers to the early genotyping of patients with NDD (or other clinical presentations) whenever a given condition is not diagnosed clinically, and subsequent establishment of a causative diagnosis with ES/GS testing, which has become more readily available and expansive. The earliest genotype-first approach is represented by the broad use of karyotyping in individuals with GDD/ID and congenital abnormalities, not clinically recognizable, which explained 10-15% of ID individuals [27,28]. Due to the ongoing advances in comprehensive phenotype analysis and cytogenomic technology, the "genotype-first" approach has increasingly become the first step within the diagnostic process in NDDs.

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Methods

The authors completed a narrative review of the literature on the rational assessment of the child with NDDs by collecting the many historical articles on the etiology of GDD/ID from prior work [5,29–34]. This search provided the historical framework. This was followed by a comprehensive review using PubMed, Medline, and Google Scholar databases led by AB to detect current papers on the diagnostic yield of next generation sequencing in individuals presenting with NDDs. Manuscripts, consisting of original papers and reviews, were reviewed for titles, abstracts, and entire texts.

The "Phenotype-First" Era Prebanding-Banding Period/FISH-Subtelomeric FISH Period

With the description of many of the now well known common congenital anomaly syndromes during the 1960s-1980s era (the "Phenotype-First" approach) researchers in the field of medical genetics developed approaches to the diagnosis of the child with these neurodevelopmental differences. The articles by Smith and Simons and Opitz et al. launched this approach and are considered classics [29,30]. From the 1970s on, research studies of individuals with NDDs helped establish a rational approach to the diagnosis of persons with these presentations [29–34]. Various investigations of individuals with GDD/ID (and more recently ASD) focused on determining a cause for the condition. These studies showed that the patients fell into six clinically-based categories [29–35]. This framework led to what was considered a more rational approach to the diagnostic assessment rather than performing all available testing. In the last three decades, working groups created by the AAP/AAN/ACMG reviewed available data and suggested focused consensus approaches to the assessment of patients [36–38]. Such consensus guidelines recommended appropriate laboratory testing and imaging based on the clinical presentation. A rational approach to a diagnostic evaluation promises to provide significant benefits to the patient, family, and practitioner. Usually the primary care practitioner weighs a variety of factors when deciding which diagnostic or screening tests to pursue on a given individual. Evaluation of the seriousness of the condition, acceptability of the test, and the importance of making a diagnosis are generally considered before proceeding with the tests. Rational evaluation is an expansion and extension of this approach specific to GDD/ID/ASD.

A thorough clinical history is of the utmost importance. A careful, head-to-toe physical examination, including a neurological assessment, and a search for skin changes should be performed. Documentation of abnormal findings, together with anthropometric measurements, is critical. Photographs, especially of the face, and video can prove very useful. Videos are an invaluable tool, documenting gait, posture, behavior characteristics, and movement disorders. The information gained from the physical examination alone can help in determining a diagnosis in about 20% of individuals or in postulating a provisional diagnosis for appropriate testing [32–35].

In the years 1960-1980, karyotyping was performed in individuals suspected of having a distinct condition, such as trisomy 21 or 18. For decades, a clinical assessment followed by standard cytogenetics of the times was the only option for several conditions as molecular testing was not available (examples in Appendix). Later, karyotyping was used more broadly in individuals with GDD/ID and multiple congenital abnormalities (the earliest "genotype-first" approach), and was successful in detecting chromosomal rearrangements larger than 5Mb, with an overall diagnostic yield of 10-15%. The identification of the first individuals with the fragile site at the bottom end of the X chromosome in 1969 [44], followed by recognition of the disorder in several other individuals throughout 1970s and 1980s with the use of cytogenetic studies, provided an extra diagnostic yield, with 1% of the GDD/ID males being affected [45]. Subsequently FISH probes, developed in 1980s-1990s, enabled the detection of smaller copy number variations (CNVs) than karyotyping and led to the recognition of many of the now classic chromosome disorders such as deletion 1p36, in the 1990s [46–50]; and to the boundaries of what is now known as the critical region of a number of disorders,

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such as Wolf-Hirschhorn syndrome [51]. Thereafter, the multiprobe FISH was shown to be highly suitable for detecting subtelomeric deletions and duplications accounting for an additional 2.5-5% of the diagnostic yield in individuals with NDDs [52–55]. To assist in this diagnostic pathway authors of previously cited articles on the rational assessment suggested algorithms [32–34].

Comprehensive phenotype analysis, including the understanding of the natural history of the condition, logically informs the development of clinical guidelines and treatment modalities. This progression from phenotype to natural history to management can be considered the central dogma of medical genetics (following the theme of the central dogma of biology, DNA→RNA→protein) [56] (Figure 1).

Even after the identification of the molecular defect, obtaining a diagnosis still depended on the clinician's expertise and ability to recognize the many hundreds of clinically recognizable disorders. Moreover, the approach could fail when the patient's characteristics did not entirely fit a known disorder, the syndrome was genetically heterogeneous, or the genetic defect was unknown. In these latter scenarios, the clinician simply did not know which gene to test, and a clinical diagnosis could not be confirmed by any molecular testing. As a consequence, the only way to identify a causative gene mutation was using Sanger sequencing [57], which, however, only allowed for the testing of one gene at a time. Thereafter, sequential single-by-gene testing was performed until a causative mutation was found based on clinical suspicion. Such an expensive diagnostic odyssey often took years, being a burden to the patients and families involved [58]. Moreover, for diagnosing patients with more than one Mendelian disorder, the phenotype-first approach would not work, and in only 7% of patients could the gene mutation causing the phenotype be identified [59]. This low diagnostic yield spawned a search for a genome-wide screening test without the a priori knowledge of the genomic locus and faster than Sanger sequencing.

The "Genotype-First" Era Cytogenomic Microarray Period

In the early 2000s, the introduction of multiplex ligation-dependent probe amplification (MLPA) and cytogenomic hybridization microarray (CMA) in both research and clinics spurred both improved diagnosis of patients with known MCA syndromes and documentation of CNVs of less than 5Mb or even smaller (<1Mb), helping both to characterize the critical regions for specific component phenotypes (seizures, face), and increasing the diagnostic yield of NDDs by another 15% [10,60]. CMA was also helpful in demonstrating the high prevalence of translocations, even cryptic, not detected by a previous karyotype combined with a given syndrome-specific FISH [61]. CMA can determine if a deletion is pure or part of a more complex imbalance, more accurately than either FISH or standard cytogenetics alone.

These new laboratory techniques also led to the identification of novel GDD/ID syndromes, such as 1q21.1 and 17q21.3 microdeletion syndromes [62–64]. All are clear-cut examples of the genotype-first approach: wide cytogenomic screening leading to a clinical characterization of a novel entity and even to a causative single gene.

Next Generation Sequencing (NGS): a Shift in the Genetic Testing Paradigm

A quantum leap in genetic testing occurred with the development of NGS whereby DNA from individuals could be screened for sequence variants in days to weeks rather than months to years [65]. Not only did this technique increase sensitivity for detection of pathogenic mutations in single genes, it enabled laboratories to develop panels to screen many genes for a given phenotype. Genetic syndromes with overlapping manifestations could be easily screened simultaneously. The convenience of NGS led to the development of wider and wider testing panels, ultimately making exome sequence (ES) analysis a reality in clinical care, considered by many to be the first tier test in the genetic assessment of individuals with NDDs [66–69]. These advances represent a paradigm shift in the assessment of NDD.

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Exome Sequencing Period

The availability of ES at lower costs and increased availability spawned the emergence of a "genotype first" approach where a genetic syndrome is diagnosed by molecular testing and not clinically. This is particularly the case when studying individuals with milder manifestations that may not be easily recognized. In addition, a broader molecular screening approach may lead to the diagnosis of individuals on the milder end of a given syndrome spectrum. This application of "genotype first" gave rise to the recognition of phenotype expansion for some of the disorders in clinical genetics, which may provide a better understanding of specific management issues on a genotype–phenotype basis [69]. The methodologies for genetic testing have been optimized for many of the syndromes. Over recent years, ES has been strongly recommended and introduced, in most developed countries, within the diagnostic setting as a first or second-tier test for individuals with MCA/GDD/ID [70–73]. Moreover, ES has been shown to be successful also in persons with ASD and epilepsy [74–76]. Overall, the use of ES in individuals with NDDs has improved the diagnostic yield up to 30-55% [10,77–84], depending on the clinical features of the population tested, year of testing, and analytical strategy [79,85–89], leaving 45% of individuals without an etiology.

As the clinical experience illustrates, the approach and yield of diagnostic testing has changed dramatically with the application of NGS from the research laboratory to the clinical setting during the decade beginning in 2010. By the end of the decade, a number of scientific journals were publishing reports of series of individuals, with similar pathogenic variants of previously known genes leading to the description of novel disease entities on a regular basis. Other studies showed that performing NGS early in the diagnostic process provided changes to the individual's management and was cost-effective, ending the diagnostic odyssey [71].

First Tier Testing for NDDs

Based on scientific data [90] and on our own experience, we propose to consider ES as a first tier test for NDDs [91]. But for the individuals who likely have a diagnosis based on the clinical presentation, such as those children with WHS or WS, the first tier test would necessarily be CMA. Other conditions in which ES should **not** be the first tier test include those disorders caused by a number of different genetic and epigenetic alterations (PWS/AS, RSS), in which the first tier test should be MS-PCR and MS-MLPA; and repeat-expansion diseases (fragile X syndrome, myotonic dystrophy) in which the first tier test should be repeat-primed PCR. Fragile-X syndrome remains a common cause of ID/ASD, and should be offered in addition to ES analysis to those individuals with a positive family history of ID and ASD (without microcephaly), and to those with a clinical-behavioral phenotype reminiscent of the syndrome. Lastly, targeted gene panels would be ideal for analyzing specific variants of genes that have suspected associations with disease (CdLS, Noonan syndromes). In summary, ES is a better choice when one is uncertain what genes need to be tested. See Figure 2, an updated algorithm summarizing this thought process and diagnostic pathway. An example illustrating the approach is in the Appendix.

In the upcoming years, WGS will likely supplant ES as the most comprehensive clinical genomic diagnostic test. However, the interpretation of and counseling for the many variants of uncertain significance (VUS) that can be found when performing ES/GS constitutes an enormous challenge.

This paradigm shift in genetic testing has created a renewed interest in phenotype, and the notion of "deep phenotyping" has emerged, and has highlighted the paramount importance of the partnership between clinicians and laboratory geneticists [94]. The techniques for exome capture and sequencing have greatly improved over time, and are relatively stable in most laboratories, but the most significant challenges are in the complexity of data analysis and interpretation of the functional impact of the detected variants and identification of the underlying affected pathway. The large amount of sequence data generated by NGS platforms has fueled the addition of bioinformatics teams into clinical diagnostic laboratories to develop data handling and analysis pipelines for such complex tests. Variants that are rare in the population and are predicted to have a functional impact on the

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gene, by altering the gene's protein coding sequence, are prioritized for analysis and human interpretation. Numerous efforts have been carried out to develop computational tools to functionally interpret both coding and non-coding genomic elements and to estimate the variants pathogenicity [95–98]. Progress made with the introduction of WGS [70] has allowed for the identification of non-coding variants and also provided more uniform coverage throughout the genome which is useful for the identification of structural variation at base-pair resolution. Overall, it seems that pathogenic de novo non-coding mutations probably account for less than 5% of exome-negative NDDs individuals [100–103].

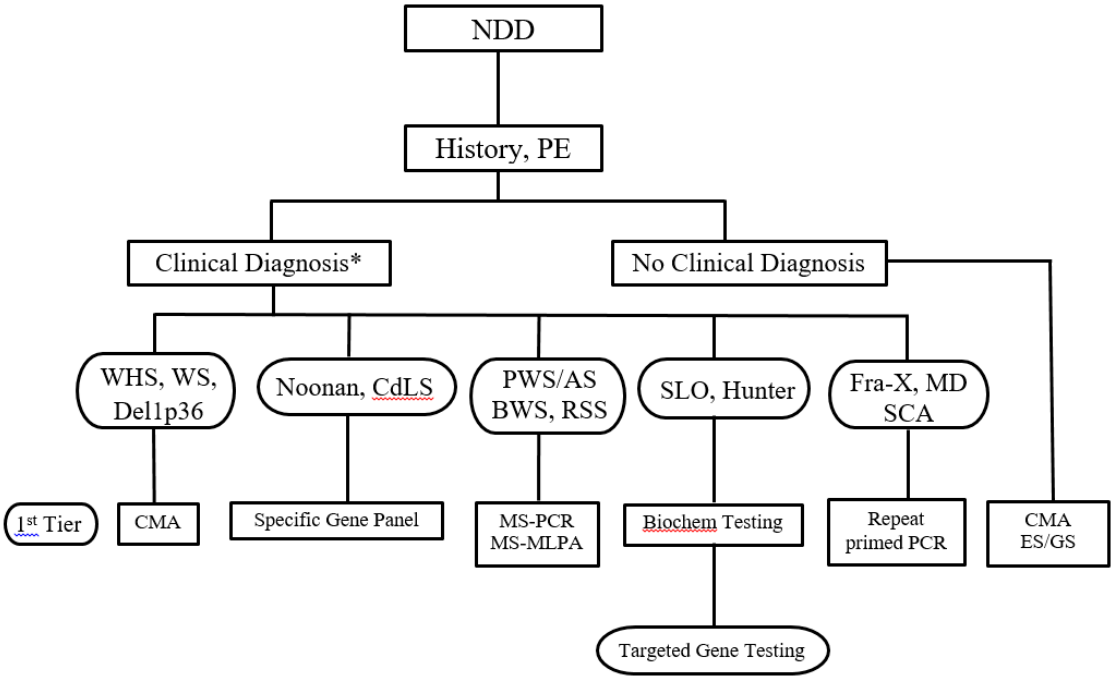

Figure 2. The algorithm depicts the current assessment of the child with a NDD. See text.

Recent studies show that both re-analysis of existing ES/GS data as well as resequencing strategies increase the diagnostic yield by 6-13% in undiagnosed NDDs individuals [103–106], and the suggested cadence of re-analysis was no more than 3 years [107–109]. A recent case report illustrates this point (see Appendix).

Notably, functional validation in specialist laboratories is essential to support the causal link between the genetic variant and the individual's phenotype, providing evidence for the variant to be considered a pathogenic variant according to the ACMG guidelines [110].

Updating clinical information before revisiting genetic data is essential. As children may not yet display the full characteristic phenotype of a given disorder, systematic reassessment of the child clinical and behavioral phenotype might show additional features implicative of a specific condition. Such evaluations may be crucial for genetic re-analysis. Assessment of the parental phenotypes is also of paramount importance, as, for instance, by assuming full penetrance of variants and apparently unaffected parents, variants in the index may be disregarded during interpretation [111]. It has similarly been found that incomplete penetrance or variable expressivity makes it hard the discovery of novel genes underlying NDDs [112].

It is reasonable to expect that other diagnostic techniques, such as methylation profiling [113], optical mapping [114], long read sequencing, or RNA sequencing [115,116], will increase diagnostic yield (for in-depth description see Appendix). Clinical utility of combining trio-ES sequencing with copy number variation sequencing in the etiologic diagnosis and clinical management of GDD is

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proven [134]. The feasibility of implementing such automated systems for re-analysis depends, however, on available budget, local infrastructure, and bioinformatic support. The budget constitutes the main obstacle both in low and middle income countries and in those countries with a national health scheme [135–137]. With not all individuals returning to the clinic for systematic follow-up, it is important to request consent from families to allow for re-analysis, also including the use of new technologies, after the initial negative or uncertain diagnostic analysis, to maximize benefits.

Due to these advances in NGS other models of care have emerged in recent years: the establishment of Undiagnosed Disease Clinics and a US clinics network [138–140] and the strategy of WGS as a first-tier diagnostic test for sick infants admitted to the NICU [86,141,143]. (see Appendix for more discussion of these approaches).

Most healthcare professionals have progressively acknowledged the benefit of NGS since it helps mitigate the "fast and furious" pace of discovery in genomics, considering the estimated 250 new gene-phenotype associations being unveiled each year [83,154]. A recent study has shown how re-analysis of trio GS data doubles the yield in NDDs, and the genetic data obtained were actionable in terms of altering management in most cases (up to 74%) [104]. Most importantly, two other recent studies showed the potential to use a single technology (long-read genomes) to accurately identify all types of clinically relevant variants, potentially qualifying long-read sequencing in the future as a first-tier test for all rare diseases [155,156], also allowing for the detection of dual pathology.

ES/GS are feasible in clinical practice and identify causal variants in a substantial proportion of persons with NDDs. But neither test can diagnose a genetic disease in a patient [94]. Clinical laboratories report variant classifications, not patient diagnoses. The ACMG guidelines recommend against making diagnostic claims or basing clinical management on genetic test result alone [157]. Diagnosis must be performed by a clinician who can assess genetic variants in the context of all of a patient's clinical features and their consistency with the full spectrum of clinical manifestations that occur in the disease being considered.

Summary and Conclusions

We have provided the historical framework and an updated approach to the rational assessment of the person with NDDs. In the child with a strong suspicion of a specific condition, the appropriate first tier testing depends on the diagnosis in question (Figure 2). The history and physical examination performed by an experienced clinician drives the choice of the most appropriate genetic testing in that situation. In the child without a clinical diagnosis we recommend ES/GS as the first step (see Appendix for further due considerations).

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Abbreviations

PE: physical examination; WHS: Wolf-Hirschhorn syndrome; WS: Williams syndrome; CdLS: Cornelia de Lange syndrome; PWS: Prader-Willi syndrome; AS: Angelman syndrome; BWS: Beckwith-Wiedemann syndrome; RSS: Russel-Silver syndrome; SLOS: Smith-Lemli-Opitz syndrome; MD: myotonic dystrophy; SCA: spino-cerebellar ataxia; CMA: cytogenomic microarray; MS-PCR/MS-MLPA: Methylation-Specific Polymerase Chain Reaction/Methylation-Specific Multiplex Ligation-dependent Probe Amplification; ES: exome sequencing; WGS: whole genome sequencing

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