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Diagnostic Yield of Exome Sequencing in Neurodevelopmental Disorders

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Introduction

Neurodevelopmental disorders affect around 3-5% of the population. These are heterogeneous in etiology and more befittingly termed "symptom complexes". The various disorders presenting with intellectual disability may be congenital or acquired, sporadic or familial, syndromic or non-syndromic. Severe neurodevelopmental disorders are mostly genetic in origin and may be due to a molecular defect at the chromosomal or single gene level or due to epigenetic abnormalities. Till date more than 450 genes have been implicated in intellectual disability. One may wonder as to what is the need for an etiological diagnosis when majority of these cases cannot be cured. However, diagnosis is a key element in the management of the patient, for explaining the course and prognosis, and for provision of appropriate care and support system. It precludes subjecting the patient to unnecessary and redundant tests and interventions. It is essential for proper counseling of the family regarding the recurrence risks and prenatal diagnosis, and also for access to research treatment protocols. The family's "need to know" has an enormous emotional impact and cannot be ignored. The elucidation of the underlying molecular abnormalities is another important step towards developing treatment strategies (Willemsen et al., 2014).

Diagnostic Approaches

Various diagnostic practice guidelines are available for evaluation of patients with neurodevelopmental disorders and consist of clinical evaluation, neuroimaging, metabolic profile, cerebrospinal fluid examination and specific genetic tests dictated by the clinico-biochemical phenotype (Michelson et al., 2011; Moeschler et al., 2014). Cytogenetic microarray which is now considered to be the first line test in the diagnosis of non-specific intellectual disability has a yield of 10-20%. This "diagnostic odyssey" lasts many years and more than half of the patients never receive an etiological diagnosis, thereby adding to the pain and disappointment besides the cost incurred by the family. In a survey of patients with rare diseases it was found that for 25% of participants, the time to diagnosis was extensive, ranging from 5 to 30 years, and during that time 40% received an incorrect diagnosis (Sawyer et al., 2016).

Whole Exome Sequencing and its Utility in Neurodevelopmental Disorders

With the advent of whole exome sequencing (WES), there came a paradigm shift in the approach to the diagnosis of rare diseases, with timely diagnosis of genetic diseases and discovery of new disease-causing genes. WES uses a high throughput sequencing technique of next generation sequencing (NGS) to sequence coding regions of all genes in the human genome. This helps to identify the causative gene/ mutation even if the clinical evaluation and supportive investigations do not provide any clue to the etiology and the causative genetic defect. This approach is being used for all genetic phenotypes with known or unknown causative genes. The study by Retterer et al. (2016) on the use of WES on 3040 consecutive cases gave a high diagnostic yield for patients who had disorders involving hearing (55%, N = 11), vision (47%, N = 60), the skeletal muscle system (40%, N = 43), the skeletal system (39%, N = 54), multiple congenital anomalies (36%, N = 729), skin (32%, N

= 31), the central nervous system (31%, N = 1,082), and the cardiovascular system (28%, N = 54). Here, we review the diagnostic yield of WES in neurodevelopmental disorders. In cases of moderate to severe neurodevelopmental disorders, different studies have reported a diagnostic yield ranging from 16-45% using WES (de Ligt et al., 2012; Yang et al, 2013; Yang et al., 2014; Sawyer et al., 2016). WES out-performs conventional approaches in the diagnosis of disorders with genetic heterogeneity with phenotypic variability and poor specificity. Besides its utility in the diagnosis of ultra-rare conditions and novel gene discovery, it also has an impact on expanding the phenotypic spectrum of already known syndromes. Most importantly, WES allows for re-assessment of data in the light of new knowledge, thus providing additional diagnostic results over years without significant extra costs. WES is an effective approach in the evaluation of cases with sporadic non-specific ID using family based strategy (child-parents trios) with yields of 35-55% (Willemsen et al., 2014). The use of WES is not only restricted to identification of these *de novo* variants but its emerging widespread use in predominantly consanguineous populations has led to the expansion of the repertoire of genes causing autosomal recessive neurodevelopmental disorders. Recently, a study in 121 consanguineous families identified pathogenic variants in 68, out of which a novel gene was implicated as causative in 30 (Riazuddin et al., 2016). The diagnostic yield of WES in cohorts with neurodevelopmental disorders in various studies has been summarized in Table 1. While the effectiveness of WES in the diagnosis of intellectual disability has been proven beyond doubt, the optimal timing of when it should be done is still debatable. Whether WES should be at the first appointment if clinically indicated or after the initial tests are normal (e.g. first tier of genes ruled out) or towards the end of the diagnostic odyssey (e.g. after extensive, possibly invasive tests have occurred), needs to be evaluated. The newer school advocating use of WES as a first-tier test have undertaken some studies using genomic tools (WES alone or with molecular karyotyping) in the diagnosis of ID patients. They have reported diagnostic yields of 32-58% (Anazi et al., 2016; Thevenon et al., 2016). Diagnostic yield as high as 91% has been reported in consanguineous families (Shaheen et al., 2016). Notably, many of the molecular defects were not suspected clinically, highlighting the power of this tool to overcome the limitations of clinical phenotyping. This has set a trend of "reverse phenotyping", whereby the success of identification of clinical recognizable ID syndromes will no longer be highly dependent on clinical expertise of syndrome recognition, causing a shift from a "phenotype first" to a "genotype first" approach. Besides, the impact on families in ending the expensive, often invasive, and stressful diagnostic odyssey cannot be overemphasized. However, in both the cases detailed phenotyping remains a very important step in the diagnostic approach to a case with a neurodevelopmental disorder and the clinician has a very important role to play in manually judging the candidate sequence variations identified by mining the exome data.

Issues Related to WES in Diagnostics

In addition to the high cost, the vast amount of data, which is a challenge for analysis and storage, is a major problem being faced. The advantage of WES is that the need of a clinical differential diagnosis is not a must and WES can identify the etiology in a case with a nonspecific or a subtle phenotype. The same advantage of covering all the genes poses challenges for analysis analysis as in some cases more than one pathogenic sequence variant may be identified or many variations of unknown significance are identified. These issues will gradually get minimized or resolved as more and more parts of the genome get annotated and databases of pathogenic and non-pathogenic variations become more comprehensive. One of the reservations which many physicians have in implementing this approach is the high cost of WES. A recent study comparing the cost of WES with conventional diagnostic approaches in a cohort of individuals with intellectual disability found that the traditional diagnostic trajectory cost was $16,409 per patient while the trio-WES cost was $3,972 only. They concluded that WES resulted in average cost savings of $3,547 for genetic and metabolic investigations in diagnosed patients and $1,727 for genetic investigations in undiagnosed patients (Monroe et al., 2016). Another concern is the inability of WES to detect copy number variations (CNVs), which cause a large proportion of intellectual disability. But in recent years, there have been many publications evaluating the utility of WES in detecting CNVs by various algorithms. These studies have reported a fair rate for detection of CNVs, 59 to 89% with the sensitivity increasing with CNVs >200kb in size (Tetreault et

Table 1 **Summary of the diagnostic yield of WES in intellectual disability (ID) in various studies.**

Study	Cohort	Timing of WES	Diagnostic yield
de Ligt et al., 2012	100 patients with severe ID	Trios WES All patients with prior extensive genetic diagnostic work up	Diagnostic yield=16%, 79 *de novo* mutations in 53 /100, Potential causative variants in 22 novel genes
Yang et al., 2013	250 patients (80% with neurological phenotype)	All patients had undergone prior genetic testing (microarray, metabolic screening and specific gene sequencing)	Diagnostic yield – 62/250 (25%), autosomal dominant =33, autosomal recessive =16, X-linked disease=9, Four probands with 2 non-overlapping diagnoses
Yang et al., 2014	2000 patients, 87.8% with neurological phenotype	All patients had undergone some prior diagnostic workup including specific genetic tests	Diagnosis in 504 (25.2%), novel variants in 58%, 6 patients with large deletions, 23 had mutations at 2 different loci
Willemsen et al., 2014	253 individuals from 234 Dutch families with unexplained ID	2 phases- diagnostic (specific genetic test, microarray, metabolic screen) and research phase (NGS in 30% of undiagnosed patients in phase I)	Phase I - diagnosis in 43 (18.4%). Phase II - pertinent/plausible diagnosis in 24 /58 (41.4%) in NGS cohort. Total diagnostic potential combining both phases = 59.8%.
Sawyer et al., 2015	>500 children from 362 families with rare genetic diseases, mainly neurodevelopmental and dysmorphism disorders	All had already received standard of care genetics evaluation	Mutations in known disease genes for 105 of 362 families studied (29%), Neurodevelopmental phenotype- yield was 31.6%
Riyazuddin et al., 2016	121 large consanguineous Pakistani families with ID	All patients had clinical +/- neuroimaging evaluation. No prior genetic test was done	Total yield - 68 families (56.2%) Novel genes - 30 families
Anazi et al., 2016	337 ID subjects, high prevalence of consanguinity	Molecular karyotyping, multigene panels, WES were used as first-tier tests, compared with standard clinical evaluation done in parallel	Standard clinical evaluation suggested a diagnosis in 16% (54/337), only 70% of these (38/54) were confirmed. Genomic approach revealed a likely diagnosis in 58% (n = 196). These included CNVs in 14% (n = 54, 15% are novel), and point mutations in remaining 43%

Note: ID – Intellectual Disability, CNV – Copy Number Variations

al., 2015; Miyatake et al., 2016). In the current situation, WES as a first-tier test in neurodevelopmental disorders appears to be an attractive option especially in families with consanguinity, multiple affected members, clinical features suggestive of a monogenic phenotype with no obvious cause and after the cytogenetic microarray is normal. More and more experience and data will provide answers to today's challenges of technical issues (like coverage), accurate interpretation of the huge data generated, incidental findings, functional validation of novel variants, and need for more robust algorithms for CNV detection.

To get the estimate of clinical utility of WES in the diagnosis of genetic phenotypes, a lot of work is needed and has already been started. The important one to be mentioned here is the Clinical Sequencing Exploratory Research (CSER) consortium which includes eighteen projects which not only are exploring clinical utility and clinical validity of clinical genome and exome sequencing, but is also looking at the ethical, social and legal implications via multidisciplinary approaches (Green et al, 2016). Similar efforts being done are 'Genome Clinic Task Force' (Fokstuen et al., 2016) and the 'SickKids Genome Clinic' (Bowdin et al., 2016). The analysis of these big studies will provide answers to the questions about appropriate use and timing of the WES-based diagnosis which is powerful but costly and has some issues which need to be sorted.

Conclusions and Future Perspective

Diagnosis of genetic disorders is an arduous and challenging task. The armamentarium of advanced genetic testing is improving the etiological diagnosis and thus helping the families. Cytogenetic microarray is considered the first tier test for evaluation of a child with a neurodevelopmental disorder. However, it should be noted that detailed clinical evaluation, appropriate imaging and biochemical investigations constitute the first step in the direction of diagnosis. As is the experience of clinical geneticists, a study of different clinical genetics centres has shown that in patients with dysmorphism, the diagnosis is achieved in the first visit in 30 to 60% of cases (Douzgou et al., 2016). However, WES offers the hope of diagnosis in many cases where there was none. In the future, whole genome sequencing (WGS) is expected to eventually replace WES and even cytogenetic microarray, as it is the single genetic test which has the potential to detect the whole spectrum of genetic aberrations ranging from single nucleotide variations to complex genomic rearrangements. However, at present and during the next few years, exome sequencing will fill the niche of being the most versatile, relatively inexpensive and hence popular application of NGS in the clinic (Tetreault et al., 2015). Its application early-on in the evaluation process of patients with non-specific intellectual disability will have a significant impact in ending the "diagnostic odyssey".

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