go treatment with a variety of rehabilitative therapies and early intervention strategies to optimize their developmental potential. NDDs could be classified on abnormalities in certain areas, such as intellectual functioning, speech, language, and fine motor skills, and might coexist with a known syndrome. In some cases, the presence of minor dysmorphism (facial and other superficial physical anomalies) or multiple congenital anomalies (MCA) might coexist with NDDs symptoms. The most common clinical features observed in NDDs patients include intellectual disability (ID) or developmental delay (DD), speech delay (SD), language delay (LD), and autism spectrum disorder (ASD).

Developmental Delay (DD)/Intellectual Disability (ID)

The developmental delay (DD) or intellectual disability (ID) is depicted by an impairment of general mental abilities, which influence adaptive functioning in conceptual domain (language, reading, writing), social domain and practical domain (organizing)6. The term DD is used for younger children who are less than 5 years of age7,8. DD/ID is estimated to occur in 1-3 of every 100 live births. ID is the latest recommended term being used worldwide to replace the term ‘mental retardation’ according to Rosa’s Law and is documented by the new International Classification of Diseases (11th revision)9. Also, DD/ID could appear as a distinct, isolated condition or coexist as part of well-defined syndromes such as autistic disorder, or X-linked ID syndromes.

Language Delay (LD)

The understanding, processing and production of communication collectively constitute language.
The language delay (LD) is more frequent in NDDs as compared to ID in the general population. The estimated range for prevalence of LD is about 5-8% in pre-school children and has been reported to co-exist with other neuropathological conditions like autism or cleft palate. LD is typically recognized by difficulty with grammar, words or vocabulary, units of words meaning, and the use of language, particularly in social contexts. LD is diagnosed by using the early language milestone scale that focuses on expressive, receptive and visual language. Children diagnosed with LD possess higher risk for learning disabilities as they have difficulties in reading, and written language, which subsequently lead to academic under achievement and lower IQ score. The difference between LD and speech delay (SD) is that LD pertains to both expressive and receptive delays, whereas speech delay is specific to speech mechanism alone.

**Speech Delay (SD)**

The mechanics of oral communication or in other words, the motor act of communicating by articulating verbal expressions, is called speech. The NDD namely speech delay (SD) is accompanied by stuttering or disfluency, articulation problems and inability to speak, observed commonly in the children younger than 5 years. The differentiating feature of SD is that not all children develop linguistic skills at the same pace or to equivalent proficiency. Similar to LD, SD is a common childhood problem that affects 3-10% of children, which could manifest with other disorders such as autism or intellectual disability, and is more frequently observed in boys.

**Autism Spectrum Disorders (ASD)**

The behavioral neural disorder characterized by selective impairment mainly in social interaction, communication, language development, and restricted or repetitive patterns of behavior (stereotyped) especially in young children is termed as ASD. ASD affects about 1 in 110 individuals in the age group of 0-3 years. It is highly heritable as compared to other types of NDDs. Furthermore, the presentation of ASD patients, are also largely variable, with symptoms ranging from mild to severe in terms of both behavioral and IQ performance.

**NDDs and Genetics**

The technological advancements like microarray comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) in the field of genetics research allowed identification of more than 400 candidate genes associated with NDDs. Some of these genes are involved in general physiological processes required for normal development, including cell adhesion, gene transcription, metabolism, synaptogenesis and chromatin remodeling. Altered gene dosage involved in these processes could disrupt the neuronal networks and could interfere with a normal brain development leading to cognitive dysfunctions.

Cytogenetics is a branch of genetics that studies chromosomes and examines the function and structure of chromosomes, and its encoded DNA that builds the genome. Conventional cytogenetic techniques such as G-banding karyotyping or FISH are very informative and have allowed better understanding of human diseases, normal phenotypic variation and karyotypic evolution. Notably, due to its genome-wide coverage and rapid turnaround time, cytogenetic analysis has been instrumental for rapid genetic evaluation of unexplained NDDs.

**G-Banding Karyotyping**

G-banding karyotyping is a conventional cytogenetic method that relies on harvesting chromosomes in mitosis. The technique involves the identification of the alternating light and dark staining bands comprising each chromosomal locus, and is useful for the identification of large chromosomal rearrangements at a resolution of 5-10 Mb. Recently, G-banding techniques are recommended as a first-tier genetic testing for specific group of patients with clinically suspected chromosome aneuploidy, such as Down, Turner and Klinefelter syndromes, or a family history suggestive of chromosomal rearrangements. This is based on the earlier observations that a sizeable proportion of NDDs cases (at a range of 4-28.4%) are attributable to chromosome abnormalities, including trisomy, subtelomeric rearrangements and balanced chromosomal rearrangements. Furthermore, subtelomeric chromosome rearrangements have been found in 6% of idiopathic severe ID patients. The diagnostic yield of routine G-banding karyotyping is observed to be approximately 3.7%. However, G-banding techniques are limited to the detection of microscopically visible chromosomal aberrations (Megabases in size), and their precise breakpoint could not be precisely delineated without further validation by ‘chromosome walking’, using probes surrounding the breakpoints by fluorescence in situ hybridization (FISH) analysis.
Fluorescence in Situ Hybridization (Fish)

G-banding karyotyping is the recent advancement, which allows unbiased view of the whole chromosomes, and is useful for genetic testing in individuals with unknown cause and no family history of NDD. However, for patients with phenotypes suggestive of specific disorder such as trisomy disorder, subtelomeric or microdeletion/duplication syndromes, a focused FISH analysis is a useful step to investigate specific syndromes. FISH analysis involves hybridization of fluorescently labeled polymorphic marker probes such as bacterial artificial chromosomes (BACs) or fosmids into the denatured DNA of metaphase chromosomes or interphase nuclei. FISH can detect submicroscopic aberrations of less than 5 Mb, and its resolution is dependent on the size of the probe in use. FISH analysis has enabled identification of many disease genes associated with congenital anomalies at the chromosomal breakpoints, such as dystrophin in Duchenne muscular dystrophy (DMD), DISC1 in schizophrenia and ATP7A in Menkes disease, as well as subtelomeric deletion syndromes. The diagnostic yield for FISH analyses in patients with ID/DD is approximately 6.8%. However, FISH could only detect known regions; therefore, it is applicable in those cases where the phenotype is suggestive of a particular disorder or having a prior knowledge of certain genomic region to be investigated.

Array Comparative Genomic Hybridization (aCGH)

The earlier failures for the need of diagnosis of NDD with high resolution and accuracy resulted in the development of a high-resolution technique namely Array Comparative Genomic Hybridization (aCGH). Its higher resolution and sensitivity allows detection of genomic changes like deletions or duplications which were previously very difficult to detect by other basic techniques including G-banding and FISH analyses. The principle of aCGH is the utilization of the cloned BACs or synthesized oligonucleotides DNA fragments covering across chromosomal loci in the genome that are spotted on the array chip. Copy numbers are determined by the differences in the hybridization patterns intensities between two differentially labeled DNA (patient and reference DNA). The resolution of the technique depends on the tiling array used. Moreover, this technique has now replaced G-banding karyotyping as the first line of genetic testing for individuals with DD/ID and has greatly improved the diagnostic yield. aCGH is a powerful approach to identify CNVs associated with NDDs, and many studies have reported the identification of recurrent microdeletions or microduplications associated with specific clinical features. Also, recent studies suggested that rare and de novo CNVs were considered to be clinically relevant and might be responsible for 15-20% of NDDs cases. The diagnostic yield of NDDs using aCGH has been observed to be four-fold higher than karyotyping. This novel classification has greatly improved diagnostic outcomes in certain group of patients. However, it is extremely challenging to identify the causative gene within the affected region for follow-up functional studies, because these regions might comprise multiple genes. However, despite the higher resolution, aCGH is unable to detect copy-neutral rearrangements or complex intra-chromosomal aberrations.

DNA Paired-End Tag (DNA-Pet) Sequencing

Large structural rearrangements such as translocation, inversion, deletion and duplication have functional roles in human traits and diseases, which have been previously characterized mainly by karyotyping, FISH and aCGH. WES recently has demonstrated its ability to detect CNVs in higher resolution based on sequencing read depth. However, neither aCGH nor exome sequencing could identify copy number neutral rearrangements, such as insertions, inversions, and translocations. For studies focusing on identification of such rearrangements, paired-end tags sequencing (PET) is the ideal approach. The principle of PET technique is to sequence only the short 5’ and 3’ tags of specific insert size of DNA fragments derived from genomic DNA in a massive and highly parallel manner. This strategy was initially applied by Korbel et al. to systematically analyze SVs of two cell lines derived from healthy individuals, which revealed extensive variation in the human genome. Another study has extended the analysis of eight individuals using cloning-based PET sequencing, and it has provided SVs map that is used as a reference for normal SVs in the population. Moreover, the unique ability of DNA-PET sequencing to detect balanced and unbalanced rearrangements encourages its utilization in mapping the breakpoints of chromosomal rearrangements for novel disease gene identification. DNA-PET sequencing is an ideal method to rapidly pinpoint the breakpoint regions of chromosomal rearrangements that segregate with disease phenotypes in familial cases, or
rare \textit{de novo} events. Potentially, DNA-PET could also be applied to examine non-syndromic NDDs patients, who usually lack a definitive diagnosis after exhaustive genetic testing.

Conclusions

A lot of advancements have been made to understand the genetic basis of NDDs. It is quite evident that these genetic consequences are the root causes responsible for variable neuropathologies suffered by affected young infants in their young as well as adult life. Further research is needed for the development of more elaborated, efficient and highly specific avenues for the management of NDDs.

Conflict of interest

The authors declare no conflicts of interest.

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