

## Clinical utility of next-generation sequencing in neurodevelopmental disorders: non-syndromic intellectual disability as a model

Nörogelişimsel hastalıklarda yeni nesil dizilemenin klinik kullanımı:  
Model olarak sendromik olmayan zeka geriliği

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### ABSTRACT

Intellectual disability (ID) refers to a diverse group of disorders with marked heterogeneity in both clinical presentation and genetic etiology. Some cases of ID are associated with distinctive clinical findings that can lead to specific clinical and molecular diagnoses. However, sporadic cases of ID also occur in which the molecular pathogenesis cannot be identified via clinical diagnosis, and the genetic etiology is often unknown. New genomic technologies such as whole-exome sequencing, in which selective sequencing of all protein-coding genomic regions is performed, have proved to be the most efficient and cost-effective approach for identifying disease-causing variants in neurodevelopmental disorders, even in small nuclear families. Successful gene discovery efforts will lead to an improved understanding of the cellular and molecular mechanisms underpinning cases of individuals diagnosed with neurodevelopmental disorders, will inform screening programs and will promote the development of novel and more effective pharmacotherapies of personalized approaches to medical management.

**Keywords:** Intellectual disability; next-generation sequencing; novel gene identification.

### ÖZ

Zeka geriliği hem klinik hem de genetik etyoloji olarak çeşitlilik gösteren geniş bir hastalık grubunu içerir. Zeka geriliği olan bazı hastalar spesifik klinik ve moleküler tanı konulmasına yardımcı olabilecek hastalığa özgü klinik bulgular gösterir. Bu tür özel klinik bulgular ile birlikte seyretmeyen zeka geriliğinde ise moleküler bozukluk klinik tanı ile ayırt edilemez ve genetik etyoloji sıklıkla bilinmemektedir. Tüm ekzom dizileme gibi genomun protein kodlayan tüm bölgelerinin dizilenmesini sağlayan yeni nesil genomik teknolojiler, nörogelişimsel hastalıklarda, çekirdek ailelerde bile hastalık nedeni olan mutasyonların bulunmasında en verimli ve uygun maliyetli yöntem olduklarını kanıtlamışlardır. Başarılı gen keşfi çalışmaları nörogelişimsel hastalıklı bireylerde altta yatan hücresel ve moleküler mekanizmaların anlaşılmasına yardımcı olacak, tarama programlarına bilgi aktaracak ve hastaların tıbbi bakımlarında hastaya özgü yeni ve daha etkili ilaç tedavilerine olanak sağlayacaktır.

**Anahtar sözcükler:** Zeka geriliği; yeni nesil dizileme; yeni gen tespiti.

Intellectual developmental disorder or intellectual disability (ID) is a neurodevelopmental disorder that is defined as an overall intelligence quotient of lower than 70, is associated with functional deficits in adaptive behavior, social skills and communication, and has an onset age of 18 years or younger.<sup>[1]</sup> Although, ID affects 1-3% of the general population.<sup>[2,3]</sup> and represents one of the main reasons for referral in clinical genetic practices, it has not received as much public attention as other common neurodevelopmental

diseases such as autism.<sup>[4]</sup> Intellectual disability can be caused by environmental and/or genetic factors.<sup>[5-9]</sup> Beyond the financial challenges, caring for a dependent with ID can have substantial social and emotional effects on a family and society.<sup>[10-13]</sup> Knowledge regarding the genetic cause of ID allows for the anticipation and treatment of associated clinical symptoms, provides information on prognosis, and prevents further superfluous and often costly testing. Additionally, it allows for the identification of

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specific treatment options or dietary guidelines and supports the testing of additional family members to determine genotyping status so that reproductive counseling may be obtained.

Unfortunately, the genetic cause of most cases of ID remains unknown.<sup>[14]</sup> Genetic causes of ID are thought to be present in 15–50% of cases,<sup>[15-20]</sup> although this number increases proportionally with severity.<sup>[2,21-24]</sup> Most severe forms of ID are due to chromosomal abnormalities or defects in specific genes. It has been shown that approximately 15% of ID cases are caused by visible cytogenetic anomalies (aneuploidies, gross deletions, inversions and rearrangements),<sup>[25]</sup> and ~15-20% are due to submicroscopic aberrations and pathogenic copy number variants.<sup>[25-32]</sup> X-linked forms are estimated to account for only 5-10% of ID cases,<sup>[33]</sup> which means that the vast majority of the underlying genetic defects remain elusive and are likely to be autosomal.<sup>[22,25]</sup> In total, approximately 2,500 genes are estimated to be involved in monogenic causes of ID.<sup>[34]</sup> The latest observations based on a parent-proband trio analysis to identify *de novo* changes in known or candidate genes for ID suggested that a significant portion of sporadic cases may be due to dominant *de novo* mutations.<sup>[35-39]</sup> More recently, Hamdan et al.<sup>[40]</sup> suggested that *de novo* mutations are the predominant cause of moderate or severe ID, on the basis of results from 41 cases of high-depth trio-based exome sequencing in patients with ID.

While autosomal recessive intellectual disability (AR-ID) is less prevalent, estimates of the contribution of recessive mutations to ID are as high as 25%, and the majority have been characterized in consanguineous families.<sup>[41]</sup> The marriage of second cousins or closer relatives, defined as consanguineous marriage,<sup>[42]</sup> is still common in many parts of the world, particularly in the Middle East and Asia.<sup>[43]</sup> In Turkey, the prevalence of consanguineous marriages has been quite high and stable in the last three decades at approximately 20-25% (ranges from 11.5% to 46%).<sup>[44-46]</sup> The children of consanguineous individuals will have more homozygous DNA than the offspring of an outbred marriage. This leads to an increased likelihood of rare, recessive, disease-causing variants being inherited from both parents. On average, first cousins have

an additional risk of 1.7-2.8% of having a child with an autosomal recessive disorder.<sup>[47]</sup> The frequency of autosomal recessive, non-specific ID is unknown. The broad genetic heterogeneity of AR-ID, which usually non-syndromic nature, and the few reported cases (most genes were detected in single kindreds) make it difficult to determine consistent genotype-phenotype correlations.

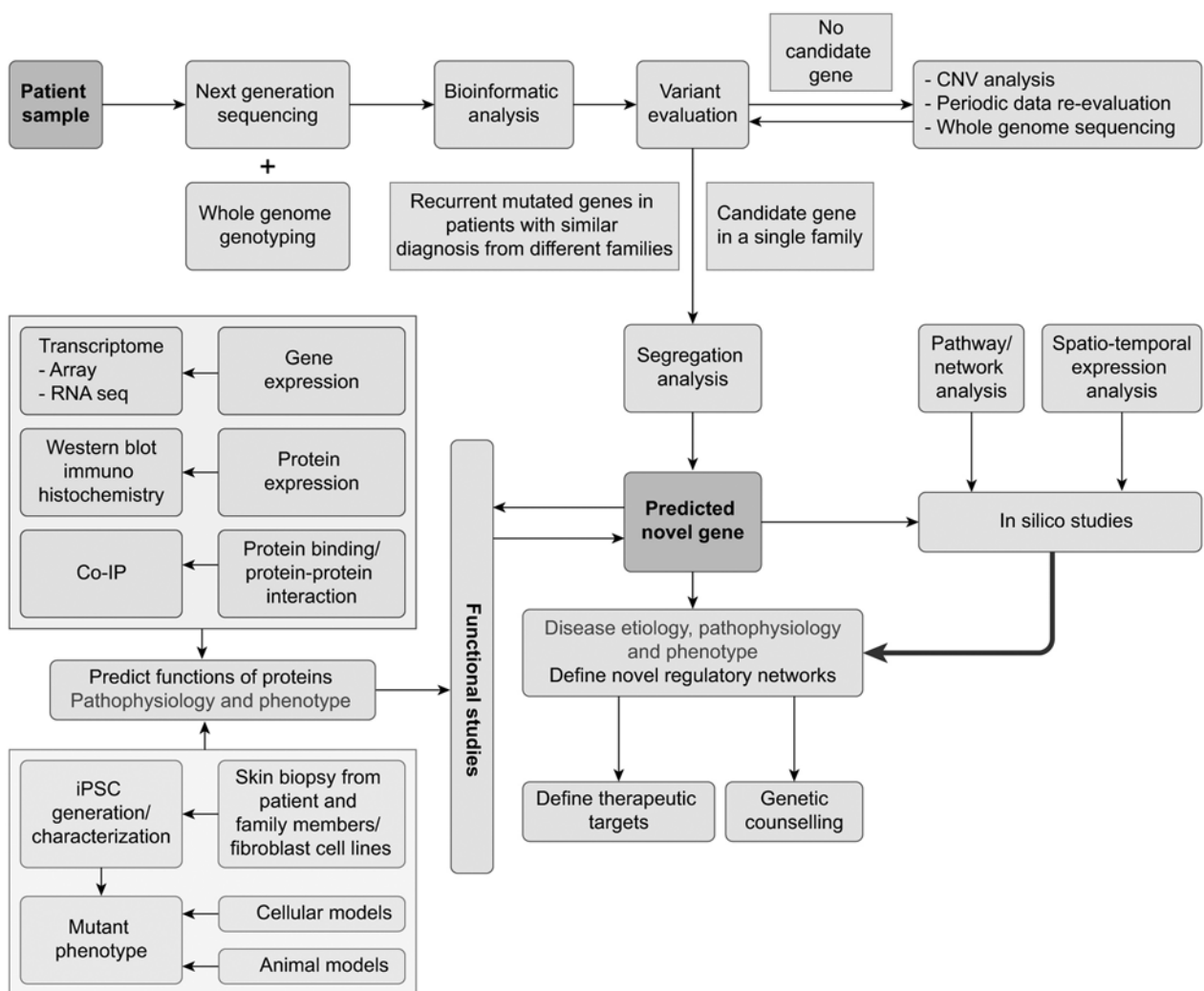
The traditional genetic testing approach for AR-ID is usually successful in identifying new genes.<sup>[48-57]</sup> This approach typically starts with targeted disease testing for mutations in known genes and consists of conventional karyotyping, especially for mosaic detection or patients with a specific medical and familial history, exclusion of fragile-X syndrome and array-based comparative genomic hybridization testing, followed by genome-wide homozygosity mapping in large consanguineous families and linkage analysis and then sequencing of genes within suspect intervals. However, positional mapping strategies (i.e., linkage analysis and homozygosity mapping) have several important limitations, for example, disease-related mutations could reside in regions of homozygosity that are too small to detect via traditional methods, particularly in probands from third cousin matings or often identify very large regions that contain hundreds of genes; and selecting relevant candidate genes can be problematic.

Whole-exome sequencing is a cost-effective and fast strategy for comprehensive mutation screening and disease-gene identification in the coding portion of the human genome.<sup>[58-60]</sup> Because it is estimated that 80% of the variants that cause Mendelian disease are located within the exome, and approximately 15% of suspected Mendelian diseases have a recessive mode of inheritance, the introduction of next-generation sequencing techniques has led to the discovery of a rapidly increasing number of autosomal recessive non-syndromic ID causative genes. This situation makes whole-exome sequencing an attractive method for investigating rare genetic variants with large effects.<sup>[61,62]</sup> In addition to mutations in *TECR*,<sup>[63]</sup> *MAN1B1*,<sup>[64,65]</sup> and *ST3GAL3*,<sup>[66]</sup> 50 putative novel autosomal recessive non-syndromic ID causative genes have been reported by Najmabadi et al.<sup>[65]</sup> However, even after next generation sequencing (NGS) testing, many patients still do not have a molecular diagnosis. For example, two large studies on the genetics of ID using whole-exome

sequencing provided a yield of 16-55%.<sup>[38,67]</sup> There may be several explanations for this finding, such as technical limitations including insufficient coverage; trinucleotide repeat expansions or low-level mosaicism, which might be responsible for the clinical symptoms; etiologic mutations that may be located in noncoding regions; or large genomic events may occur. Alternatively, whole-genome sequencing is considered to be the most comprehensive form of genetic testing currently available.<sup>[68]</sup> More recently, Glissen et al.<sup>[69]</sup> performed whole-genome sequencing on 50 patients with severe ID and their unaffected parents with an average genome-wide coverage of 80-fold, and a diagnosis was made in 42% of the patients. Whole genome sequencing is also not without its limitations, such as the fact that

not all areas of the genome may be captured and analyzed, the control datasets for non-coding variants are less mature and still expensive than the whole-exome sequencing.

Numerous challenges are inherent in the identification of rare and common variants that have a role in IDs. One such challenge is the interpretation of pathogenic variants, including those derived from well-known disease-causing genes.<sup>[70]</sup> Further functional testing should be performed on novel gene variants with the aim of enhancing our understanding of the molecular basis of the disease.<sup>[60,71-77]</sup> Increased knowledge of the genetic and cellular mechanisms that cause ID could lead to the development of novel treatment options (Figure 1).



**Figure 1.** Novel gene identification steps in a patient with intellectual disability. A systemic approach is used to identify candidate variants and determine their pathogenicity.

Regardless of advances in molecular technology, the currently identified mutated genes are responsible for only a small fraction of non-syndromic ID cases and the remaining disease-causing genes have not yet been identified.

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