CONTEMPORARY APPROACH TO DIAGNOSIS OF GENETIC CAUSES OF INTELLECTUAL DISABILITY

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Abstract

Intellectual disability is a lifelong debilitating developmental disorder with important genetic contribution, which remains challenging to investigate due to high clinical and genetic variability. Finding the etiologic diagnosis of ID, however comes with great benefits for patients and their families, therefore establishing a genetic diagnostic pathway with right combination and succession of diagnostic tools is crucial for both prevention and appropriate treatment and/or rehabilitation of patients with ID. New diagnostic tools in genetics such as array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) have much higher detection rate for genetic aberrations responsible for ID and have enormous potential to shorten the path to diagnosis, as early diagnosis is a cornerstone for medical and non-medical management of persons suffering from ID.

Keywords: intellectual disability, aCGH, NGS, genetic testing

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Introduction

Intellectual disability (ID) presents the most common developmental disorder. It is a lifelong disability characterized by substantial impairment of intellectual functioning and adaptive behavior (conceptual, social and practical skills), with onset in infancy or early childhood (1, 2). Prevalence of ID varies greatly worldwide, however most studies estimate it affects between 1% and 3% of general population (3). Lifetime costs (direct and indirect) to support individuals with ID are large, estimated to be an average of approximately $1 million per person in the USA (4).

ID can be described by severity of impairment or by the presence of additional clinical signs (syndromic/non-syndromic). For classification of severity of ID terms mild (IQ of 55 – 70), moderate (IQ of 40 – 50), severe (IQ of 25 – 40) and profound (IQ of less than 25) are used. When there is involvement of other organs, presence of malformation or typical clinical signs ID is defined as syndromic. Non-syndromic ID is intellectual disability that occurs either in absence of other clinical signs or is associated with minor physical, neurological and/or metabolic abnormalities, such as autism spectrum disorders, attention deficit hyperactivity disorder, epilepsy and neuromuscular deficits (2).

ID is a result of the interplay between environmental and genetic causes, especially during prenatal life (5–9). Environmental causes include prenatal infections, maternal conditions (e.g., diabetes), exposure to harmful substances during pregnancy (e.g., alcohol, drugs, environmental chemicals), complications during birth (e.g., perinatal asphyxia), preterm birth and acquired brain injury (2).

In light of ever improving antenatal and postnatal care, different genetic abnormalities emerge as an important cause of ID. Down syndrome – trisomy 21 that is result of chromosomal abnormality is still one of the most common causes of ID/DD with the estimated frequency of 1 in 800 live births (10) despite of significantly improved...
ИП со процентата фреквенција од 1 кај 800 живородени деца (10), и покрај значително подобрените можности за пренатален скрининг. Втората најчеста генетска причина за појава на ИП се сместа дека е синдром на фрагилен Х-хромозом, со фреквенција од 1,4 на 10.000 мажи и 0,9 на 10.000 жени од вкупното население (11).

Големото подобрување на генетската етиологија на ИП е последица на две нови методи воведени неодамна, на низата компаративни геномски хибридензации (aCGH) и на секвенциранието на следната генерација. Имено, aCGH откри нова категорија на геномски мутации, субминискулски деленици и удвојувања на ДНК, таканаречена копија на варијација на број (CNV), кои не се откривале со каротипизацијата. Покрај тоа, секвенцирањето на следната генерација открива огромна генетска хетерогенитет на моногенски причини за ИП со повеќе од 800 гени веќе поврзани со ИП (2). Понатаму, и aCGH и NGS придонесоа за точна парадигмат во ИП, билдејќи методологиите покажаа голем дел од почново доминантните мутации во спореднички случаи на ИП (12). Коплекскот генетска архитектура подразбира очигледна потреба да се промени дијагностичката патека за ИП. Раната дијагноза на ИП е неопходна за избегнување на непотребните или излишни дијагностички тестови коишто честопати резултираат со дијагностичка однос иова; овозможува рафинирани опции за претман и подобрување на разбирания на прогнозата, управување со симптомите или надзор за познатите комплексации, процена на повторување на потенцијалните ризици и примарната превенција, како и можност за коуправување на соодветни пациенти во контекст на медицински дом со цел да се обезбеди најдобро здравствена состојба, социјална и здравствена заштита за детето и семејството (13).

Целта на овој труд е во кратки роти да ги разгледа тековните релевантни генетски дијагностички алатки и да предложи генетски дијагностичка патека за ИП.

Дијагностички алатки
Важни карактеристики на дијагностичките алатки се клиничката важност и корист. Додека клиничката важност е мерка за тоа колку точно тестот ја детектира или предвидува клиничката состојба на односното лице, кли-}

screening possibilities prenatally. Second most frequent genetic cause of ID is considered to be Fragile X syndrome with the frequency of 1.4 per 10,000 males and 0.9 per 10,000 females in the total population (11). Major improvement of genetic etiology of ID/DD is a consequence of two novel methodologies introduced recently, array comparative genomic hybridization (aCGH) and next-generation sequencing. aCGH namely revealed a new category of genomic mutations, submicroscopic deletions and duplications of DNA, also called copy number variation (CNV) not detectable by karyotyping. Moreover, next generation sequencing revealed immense genetic heterogeneity of monogenic causes of ID/DD with more than 800 genes already associated with ID/DD (2). Furthermore, both aCGH and NGS contributed to the “de novo” paradigm in ID, since methodologies demonstrated a large proportion of de novo dominant mutations in sporadic cases of ID (12). The complex genetic architecture implies the obvious need to modify diagnostic pathways of ID. Early diagnosis of ID is namely necessary for the avoidance of unnecessary or redundant diagnostic tests often resulting in diagnostic odyssey, refined treatment options and improving understanding of prognosis, symptom management, or surveillance for known complications, estimation of recurrence risks and potential primary prevention as well as opportunity for co-management of appropriate patients in the context of a medical home to ensure the best health, social, and health care services satisfaction outcomes for the child and family (13).

The purpose of this paper is to shortly review the current relevant genetic diagnostic tools and to propose the genetic diagnostic pathway for ID/DD.

Diagnostic Tools
Important characteristics of diagnostic tools are clinical validity and utility. While clinical validity is a measure of how accurately the test detects or predicts the clinical condition of interest, clinical utility
Karyotyping is a method of classical cytogenetics that has been utilized in clinical research laboratories for more than 50 years and is still the most readily accessible method for detecting chromosomal abnormalities (14). However, karyotype cannot detect subtle chromosome abnormalities that are at the limit of light microscopy (overall resolution is 5 Mega bases) and does not detect submicroscopic rearrangements or might potentially wrongly characterize chromosomal rearrangements as balanced, when they are not at the DNA level (15,16). Studies showed that, when patients with Down syndrome were excluded form evaluation, karyotyping identified chromosomal abnormalities in less than 3% of individuals (16). Balanced rearrangements and low-level mosaicsms are not detected by aCGH, but they are rarely (in <1% of cases) responsible for intellectual disability (17). Chromosomal G-banded analysis is still the method of choice if aneuploidy syndrome is suspected (e.g. Down syndrome) and might still be considered in the case of a family history of chromosomal rearrangement or multiple miscarriages.

Karyotype is widely available and reimbursed genetic test with a cost around 400 EUR in European countries.
Array comparative genomic hybridization (aCGH)

Array comparative genomic hybridization presents a new cytogenetic technique, which has the ability to simultaneously detect aneuploidies, deletions, duplications and/or amplifications of any locus represented on an array (18). It has much higher resolution (<5 Mega bases) to detect copy number variants compared with karyotype and it does not depend on staining and visual resolution limits (19). Because of higher resolution it can also detect submicroscopic chromosomal abnormalities (18).

Array comparative genomic hybridization studies have identified numerous recurrent CNVs leading to description of novel ID syndromes and identification of causative genes in these CNVs. It has been estimated that the introduction of aCGH led to a 15% to 20% increase in etiologic diagnosis for patients with ID and associated facial dysmorphisms of unknown cause (20). Moreover diagnostic yield is high even in milder forms of DD/ID (21).

Array comparative genomic hybridization is usually not associated with therapeutic or curative interventions for the cognitive dysfunction. However, the test results may impact on other comorbid conditions that could not be predicted on physical examination alone and may have important reproductive implications. Moreover, it has been recently reported that maternal quality of life improved when aCGH succeeded to clarify etiologic diagnosis in disabled child (22).

As aCGH interrogates with the whole genome, there is a risk of finding additional genetic abnormalities - incidental findings, not related to the diagnostic hypothesis (23). Therefore, genetic counselling before and after genetic testing is important. Array comparative genomic hybridization has been introduced in most developed health systems but is still unavailable or not reimbursed in several South European countries. The cost depends on the resolution of the arrays and usually ranges from 500 to 900 EUR.
Секвенцинарење на следната генерација
Отомното количество на време и ресурси потребни за секвенцинарење на првото човечки геном во рамките на Проектот за човеков геном го стимулираше развојот на побрз, попродуктивни и поевтини техноло- ги, сега познати како секвенцинарење на следната генерација (NGS). NGS претставува голем чекор напред, бидејки во исто време може да врши изјави за повеќе милиони секвенцинарирања, со што драстично го забр- зува процесот на секвенцинарење. Како резултат на тоа, неколку гени, сите егзоми во геномот, па дури и целиот геном може да се анализира во еден генетски тест по разумена цена.

Постојат три главни пристапи во NGS за количината на истраѓања на податоци – NGS со примена на панел, кои се созојат од гени кои веќе биле поврзани со одредени болести, секвенцинарење на целиот егзом (WES) и секвенцинарење на целиот геном (WGS).

Високата стапка на де ново доминанти мутации се демонстрира со секвенцинарење на егзомот со примена на пристапот погодено дете плус родители (24). Истиот пристап предложи високи, 44 - 45% дигано-генетички резултати во германската група на пациенти со ИП (25). Насочената (панел) NGS-анализа со ИП постигна само помал резултат од 11% за различа од резултатите на aCGH.

Со примената на WGS беа достигнати дополнени 42% повеќе од aCGH и од секвенцинарирањето на егзомот, и кумулативен резултат од 62% (26). Како што беше случајот со aCGH, случајни наоѓа може да бидат откриени за време на NGS-тестирањето, во зависност од тоа кој пристап NGS ќе се приме-ни.

Дигано-генетички потенцијал донекаде е ограничен поради недостаток на информации за фракцена и патогеност (на пример, варианти од непознато значење) на низа варијан-тити (25, 27).

Постои широка варијабилност во достапнос- та и надоместот за NGS во европските земји. Тестот е воведен како дел од клиничките дигано-генетички услуги, на пример, во Холандија и Словенија. Цената на NGS варира во зависност од пристапот и се движи од 1000 - 1800 евра за пациент.

Next-generation sequencing
The vast amount of time and resources needed to sequence the first human genome in scope of The Human Genome Project stimulated the development of faster, higher throughput and cheaper technologies, now known as next-generation sequencing (NGS). NGS represents a major step forward as it can carry out thousands-to-many millions of sequencing reactions at the same time, thus drastically speeding up the process of sequencing with Sanger. Consequently, several genes, all exomes in the genome or even whole genome can be analyzed in one genetic test at a reasonable cost.

There are three main approaches in NGS regarding the quantity of investigated data – NGS using panels that comprise genes that have already been linked to certain disease, whole exome sequencing (WES) and whole genome sequencing (WGS).

A high rate of de novo dominant mutations have been demonstrated by exome sequencing using affected child-parents trio approach (24). The same approach proposed a high, 44-45% diagnostic yield in the German cohort of the ID patients (25). Targeted (panel) NGS analysis of affected ID patients only reached lower yield of 11% beyond the yield of aCGH.

Using WGS further 42% diagnostic yield beyond aCGH and exome sequencing and cumulative yield of 62% was reached (26). As was the case with aCGH, incidental findings might be revealed during NGS testing, dependent on the NGS approach (panels/WES/WGS) used.

Diagnostic potential is somewhat limited due to the lack of information on frequency and pathogenicity (e.g., variants of unknown significance) of sequence variants (25, 27).

There is wide variability of the availability and reimbursement of NGS in European countries. The test has been introduced in the clinical diagnostic service e.g. in Netherlands and Slovenia. The cost of NGS varies according to the approach (panel/exome sequencing) and ranges from 1000 – 1800 EUR for a patient.
**Genetic diagnostic pathway**

The approach to genetic diagnosis still remains based on the solid clinical evaluation of ID/DD which should firstly exclude non-genetic causes. Furthermore, clinical evaluation directs genetic evaluation to targeted genetic tests in the case of specific diagnosis (e.g. trisomy 21 - chromosomal syndrome (aneuploidy), Fragile X syndrome or Prader-Willi syndrome) or hypothesis free search for genetic abnormality using genomic tests like aCGH and NGS (Fig 1).

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**Слика 1. Патека за генетска дијагностика на ИП / Figure 1. Genetic diagnostic pathway for ID/DD**

Legend to Fig 1. *fragile X testing might be considered in males with moderate to severe ID, DD/DD – developmental delay / intellectual disability, aCGH – array comparative genomic hybridization, NGS – next-generation sequencing
Clinical evaluation should include comprehensive medical history (prenatal, birth and developmental history) with a three generation family tree and comprehensive physical examination (other affected organ systems, dysmorphologic, neurologic and behavioral abnormalities).

If no specific diagnosis results after clinical evaluation, aCGH is the method of choice for unexplained ID/DD. If no mutation is found, fragile X testing might be considered especially in males with moderate to severe ID.

Chromosomal G-banded analysis might still be considered in the case of a family history of chromosomal rearrangement or multiple miscarriages. If still no diagnosis has been established NGS is indicated. If WES or WGS is used as NGS methodology, both comprehensive set of known genes related to ID/DD as well as metabolic genetic causes for ID can be screened for mutations.

Conclusions

While the knowledge of genetic etiology in ID/DD has significantly increased recently, there are still several challenges in implementation of new genomic methods in the clinical practice. These include limited evidence of clinical utility of aCGH and NGS, lack of diagnostic standards and guidelines - especially in NGS, limited access to expertise and testing in domestic health systems or cross border health care and lack of reimbursement for new genomic tests. Nevertheless, due to high familial and societal burden of ID one hand and benefits of early diagnosis on the other, implementation of new diagnostic methods in health systems is warranted.

Conflict of interests

Authors declare no conflict of interests.

References