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Establishing genetic diagnosis of intellectual disability in children: diagnostic yield of various genetic approaches

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Background. The aim of our investigation was to examine aetiology of intellectual disability / developmental delay (ID / DD) in children referred to the Centre for Medical Genetics at the Vilnius University Hospital Santariskiu Clinics during 2009 and to evaluate the diagnostic yield of current genetic approaches.

Materials and methods. In a retrospective investigation, medical records of 217 patients younger than 18 years of age were reviewed with a focus on the family history and pedigree, personal history, physical examination, imaging and laboratory diagnostics. Patients with established genetic diagnosis were compared with cases without identified disorders. Aetiological structure of all cases was explored, as well as factors influencing the diagnosis of genetic predisposition and the yielding of the genetic methods for investigation of patients with ID or DD available in 2009.

Results. Genetic diagnosis was established for 88 (40.5%) patients. The diagnostic yielding of conventional karyotyping was 18%, molecular karyotyping 12.4%, metabolic testing 4.1%, FISH 2.3%, molecular genetics 0.9%, in 2.8% of patients the fetal alcohol syndrome was diagnosed.

Conclusions. Currently the most effective are conventional karyotyping and molecular karyotyping techniques, showing that chromosomal alterations are the most common cause of ID / DD. Mostly the diagnosis is established in severe cases of ID / DD with congenital anomalies and dysmorphic features. Metabolic testing is especially effective if suggestive clinical features of metabolic disorders are present. The low yielding of molecular genetics methods indicates the need of their integration into diagnostics of ID / DD in Lithuania.

Key words: children intellectual disability, aetiology, genetic approaches

INTRODUCTION

Intellectual disability (ID) is described as a development disorder, manifesting in children (<18 years of age), and is characterized by the dysfunction of intellectual and adaptive abilities, including a range of conceptual, social and practical skills (1). It is one of the main disabling conditions in present-day environment, and is thought to affect 1–3% of the population (2). Generally, ID is concluded when the IQ score of children

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is observed to be below 70, i. e. more than two deviations lower than the population medium of 100 (3). In children (up to 5–7 years of age), where standard psychometric analysis cannot be carried out, a definition of developmental delay (DD) is more common (4).

Intellectual disability can be caused by a variety of factors. However, at present specific aetiology can only be described in about 50% of the cases, while the rest remains elusive (5). Combined data from a number of related studies suggest that about 17–47% of all ID / DD cases could be attributed to genetic abnormalities (6). These estimations vary in respect to means of analysis employed, as well as between countries of different developmental status.

Aetiology of complex ID / DD disorders is crucial to understand in order to determine the basis of the disease, its prognosis, risk of complications and familial recurrence, as well as to prevent any unnecessary medical testing, establish the most appropriate course of treatment and support social integration of individuals with intellectual disability. The aim of our investigation was to examine aetiology of ID / DD in children referred to the Centre for Medical Genetics at the Vilnius University Hospital Santariskiu Clinics (VUH SC) during 2009 and to evaluate the diagnostic yield (i. e. detection rate of pathogenic abnormalities) of current genetic approaches.

MATERIALS AND METHODS

In a retrospective investigation, medical records of 217 patients younger than 18 years of age were reviewed. All these patients were consulted at the Centre for Medical Genetics at VUH SC by clinical geneticist concerning ID or DD conditions, between 1 January and 31 December 2009. The medical records of all patients were reviewed with a focus on the family history and pedigree, personal history, physical examination, imaging and laboratory diagnostics.

The data from the family history and pedigree were evaluated in order to determine if ID / DD is sporadic or familial. In familial cases the mode of inheritance was evaluated. When considering the personal history, the known causes of ID / DD were searched (prenatal, e. g. infectious, toxic agents, as well as perinatal and postnatal, e. g. prematurity, cerebral trauma). The Apgar scores, anthropometric parameters of the newborn, congenital malformations, natural history (reaching the main milestones) were registered. In accordance with physical evaluation, the detailed phenotype of patients was observed and the following types were distinguished: normal phenotype, minor anomalies and congenital anomalies of body systems. We have also evaluated the diagnostic yielding of different genetic methods to determine the aetiology of ID / DD, with a focus on the following types of genetic tests: karyotype, FISH, molecular karyotyping, molecular genetic testing and biochemical analysis.

According to physical examination and laboratory diagnostics results, ID / DD cases were grouped into the following aetiological categories based on recommendations of Wellesley et al. (7): caused by chromosomal abnormalities (microscopically visible, unbalanced chromosomal abnormalities), microdeletions / microduplications (for all submicroscopic chromosome abnormalities), teratogenic factors (for known teratogens and prenatal infections), familial cases (for familial disorders), syndromic (for recognized non-familial, non-chromosomal cases or for patients with ID / DD and abnormality within at least one body system or at least 5 minor anomalies), associated with multiple anomalies (a patient had two or more abnormalities within different body systems, or at least one such abnormality plus at least 5 minor anomalies) or isolated ID / DD (where no specific phenotypic features nor genetic pathology could be identified).

Patients with established genetic diagnosis were compared with cases without known clinical diagnosis. Aetiological structure of all cases was explored, as well as factors influencing the diagnosis of genetic predisposition and the diagnostic yielding of the genetic methods for investigation of patients with ID or DD available in 2009.

All data were analyzed using the SPSS 17.0 programme. Descriptive statistics were computed for demographic information. By using cross tabulations, we analyzed univariate associations between variables. Chi-square test was used for statistical analysis. P values <0.05 were considered as significant.

RESULTS

Out of 217 patients, 134 (61.8%) were males, 83 (38.2%) females. 54 (24.9%) patients were younger than 1 year, 110 (50.7%) were between 1

and 6 years and 53 (24.4%) were 7 years of age or older. 161 (74.2%) patients were found to have DD, 27 (12.4%) had mild ID, 6 (2.8%) had moderate ID and 6 (2.8%) were diagnosed with severe ID. Most cases were sporadic (165, 76%), however, a small percentage had a family history of ID (25, 11.5%). In 27 (12.4%) cases the pedigree was not available.

Most of the patients observed were full-time births (164, 75.6%), 43 (19.8%) were born prematurely and 9 (4.1%) were late births. 166 (76.5%) had normal weight at birth, 35 (16.1%) were below and 15 (6.9%) were above normal birth weight. According to the Apgar evaluation, most children were scored 8 to 10 (136, 62.7%), 14 (6.5%) received a score of 7, 11 (5.1%) were scored 4 to 6, 1 patient (0.5%) received 3. Complicated labour was stated in 110 (50.7%) of all cases. Most children were single-foetus births (210, 96.8%), 6 (2.8%) were of twin and 1 (0.5%) of triplet births.

Most mothers were between 18 and 35 years of age at the time of delivery (199, 91.7% cases), 3 (1.4%) women were younger than 18, and 15 (6.9%) were older than 35 years. 152 (70%) mothers were healthy during pregnancy and labour, 32 (14.7%) had diagnosed infection, 10 (4.6%) had cardiovascular disease, 9 (4.1%) were associated with psychiatric disorders, 5 (2.3%) had endocrinal pathology, 5 (2.3%) were with urogenital pathology, 2 (0.9%) had respiratory system pathology, 1 (0.5%) had oncological disease, 1 (0.5%) was with autoimmune disease. 22 (10.14%) mothers were reported to have cigarette smoking during pregnancy, 5 (2.3%) used alcohol, 1 (0.5%) was narcotic addicted.

The statistical analysis of data from the family history, pedigree and personal history did not reveal any significant differences between the groups of patients with unknown and established genetic diagnosis, but was essential for choosing the strategy of genetic investigation.

Large proportion of patients (88, 40.6%) had one or more minor anomalies, 19 (8.8%) patients had congenital anomalies, 30.4% had both minor anomalies and congenital anomalies, and the phenotype was normal in 44 (20.3%) patients. Multiple congenital anomalies occurred most commonly (31 patient, 14.3%), subsequently CNS malformations were present in 26 patients (12%) and heart defects in 19 patients (8.8%). 34 (15.7%) patients had epilepsy.

Aetiological classification of all investigated ID / DD cases is demonstrated in Fig. 1. The majority (33.6%) of cases was found to be syndromic, 18% were caused by chromosomal abnormalities, 11.1% were microdeletions / microduplications, 5.5% of all cases were associated with multiple congenital anomalies, 2.8% were influenced by teratogenic factors. In 29% of all cases no specific phenotypic or genetic features could be identified, therefore these were considered as isolated ID / DD cases.

The potential of different genetic tests is summarized in Fig. 2. Genetic diagnosis was established for 88 (40.5%) patients, however, genetic testing was inconclusive for the rest 129 (59.5%) patients. In 39 (18%) cases a genetic condition was identified after karyotyping analysis, 27 (12.4%) cases – molecular karyotyping, 9 (4.1%) – biochemical analysis, 5 (2.3%) – FISH, 2 (0.9%) – molecular genetic

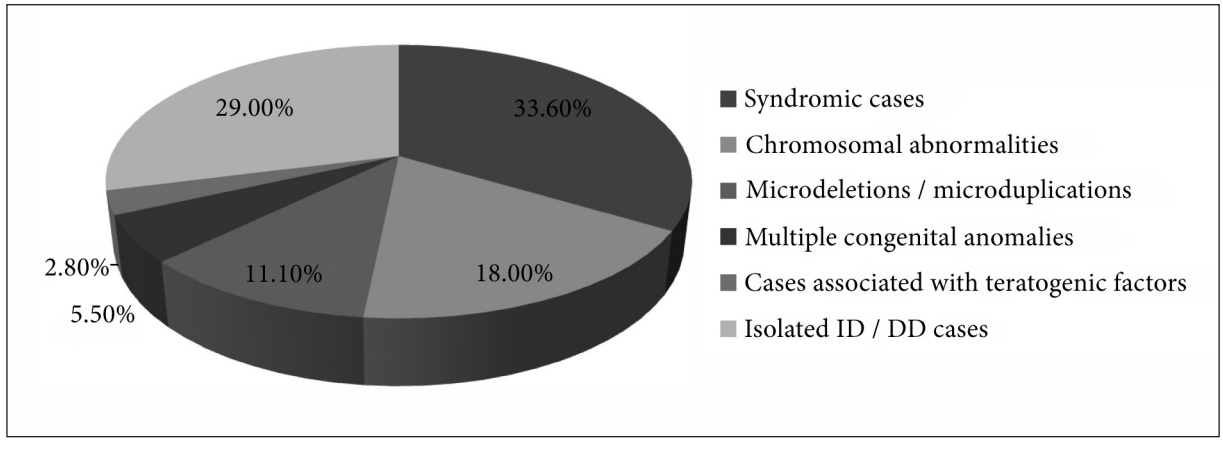

Fig. 1. Aetiological classification of all investigated ID / DD cases

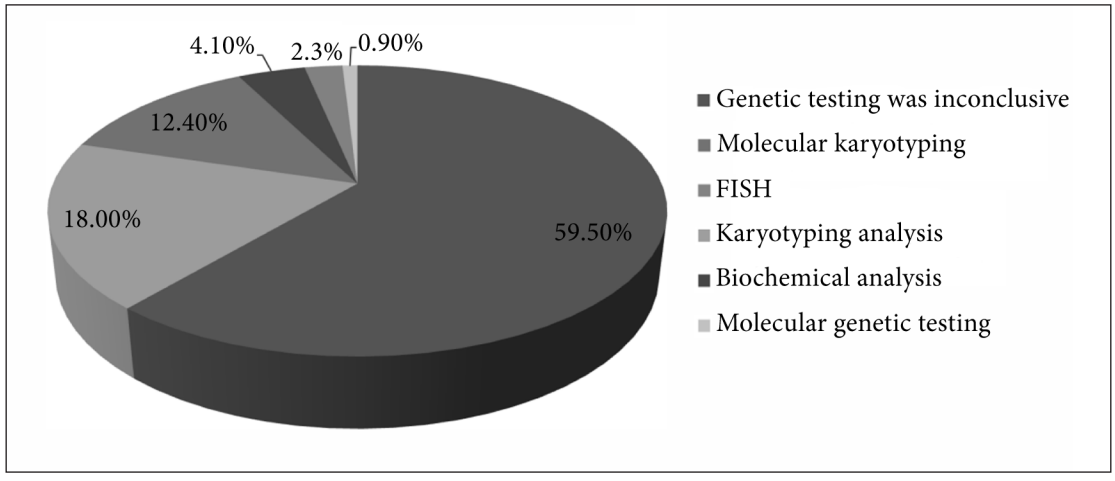

Fig. 2. The potential of different genetic tests

testing. After phenotypic examination and evaluation of anamnesis, 6 (2.8%) patients were diagnosed with foetal alcohol syndrome.

Phenotypic observations of all cases are summarized in Fig. 3. Patients without established genetic diagnosis more often did not exhibit any specific phenotypic features, or only had certain minor anomalies ($p = 0.000$). The genetic diagnosis was more often established in patients with congenital anomalies and minor anomalies ($p = 0.000$), no individuals in this group were observed to have a normal phenotype.

Interestingly, genetic diagnosis was more often established in female patients ($p = 0.001$). The patients younger than 1 year of age more often had a genetic diagnosis than the older patients ($p = 0.000$) (Fig. 4) and it was most often established after the karyotype test. For patients older than 1 year, the molecular karyotyping test was the most informative (46.7%, $p = 0.001$).

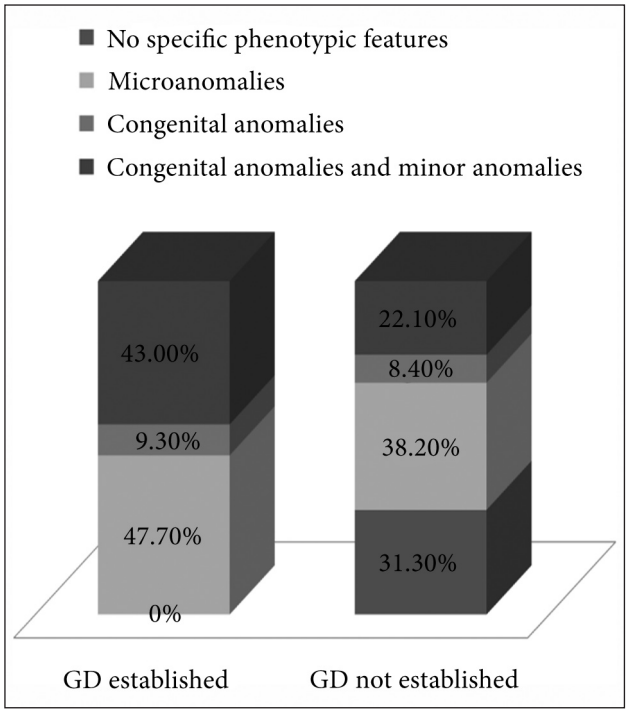

Fig. 3. Phenotypic observations of patients with established and not established genetic diagnoses (GD)

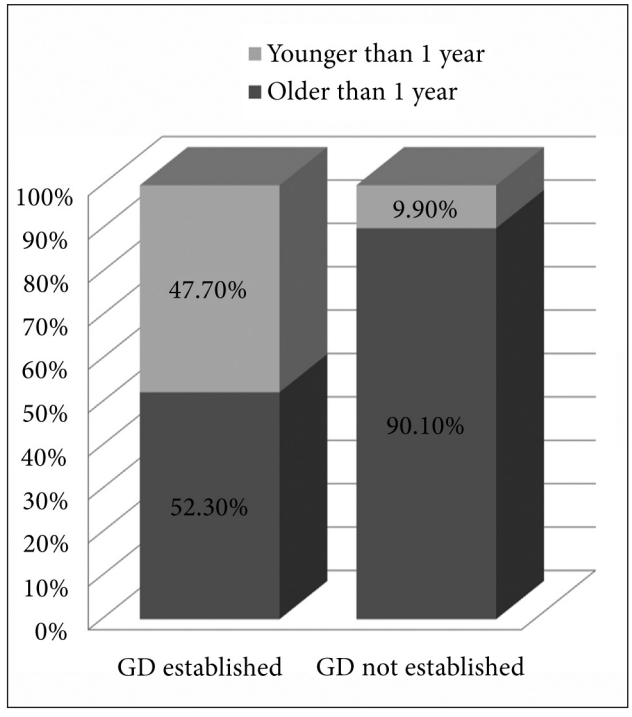

Fig. 4. The establishment of genetic diagnosis (GD) depending on age

DISCUSSION

ID and DD are caused by a variety of factors, ranging from genetically predisposed to environmental effects. Lately, however, genetic aetiology has been determined for more and more ID / DD cases, especially in developed countries. Within our investigation we have discovered genetic testing to be informative only for a percentage (40.5%) of all ID / DD patients, which is in line with similar studies where genetic predisposition is reported in 17–47% of cases (5, 10). According to our results, the genetic diagnosis was most commonly established in infancy and in more severely affected patients, when besides ID / DD, congenital anomalies and dysmorphic features were present. In these patients, chromosomal anomalies were detected most commonly, making the conventional karyotyping and molecular karyotyping currently the most effective diagnostic test in cases of ID / DD.

Pathogenic chromosomal abnormalities detected by first karyotyping accounted for 18% of all cases, which is in the range from 4 to 28% from various studies (8). The vast majority of these cases was trisomy 21 (32 cases), which accounts for 14.7% of all cases selected in this study and represents the most common known cause of ID / DD, occurring in approximately 1 out of every 700–1 000 births. Down syndrome is easily recognizable due to its association with specific chromosomal anomaly and pronounced morphological and behavioral abnormalities. Unfortunately, high percentage of intellectual disability cases does not manifest in well defined phenotypic signs, and cannot be easily assigned to one or another predisposing condition (1). However, karyotype analysis is effective in detecting chromosomal gains and losses of more than 5–10 Mb as well as balanced rearrangements, including reciprocal and Robertsonian translocations, inversions and triploidies.

The second most common cause of ID / DD was clinically unrecognized microdeletions and microduplications, detected by molecular karyotyping (array comparative genome hybridization, array-CGH). This technique identifies copy-number variations (amplifications / deletions) across the entire genome at high resolution. The use of array-CGH for clinical testing of patients with ID / DD and congenital anomalies has provided a tremendous improvement on the efficiency of mapping chromosomal imbalances. Array-CGH permits the identification of novel microdeletion / microduplication syndromes, the identification of reciprocal products, the expansion and characterization of associated phenotypes, the elucidation of the underlying genomic etiology of previously well-known conditions and has accelerated the pace of discovery and confirmation of genes, which are implicated in the manifestation of both contiguous genes and monogenic conditions. According to Siggberg et al. (11), the diagnostic yielding of molecular karyotyping after a negative karyotype is 10.8% with low-resolution arrays and 15.8% with high-resolution arrays. Current guidelines recommend the array-CGH testing as the first genetic test for patients with ID / DD or autism (12), detecting >99% of all pathologic chromosomal abnormalities. Karyotype analysis is currently recommended over the array-CGH only for patients with obvious chromosomal syndromes (e. g. trisomy 21), in case of known family history of chromosomal rearrangement or genotype-phenotype inconsistency.

Clinically recognizable syndromes were diagnosed to 5% of patients. 2.8% of cases were teratogenic syndromes (mainly fetal alcohol syndrome). This value overpasses 0.5–1% of fetal alcohol syndrome cases reported in several studies analyzing patients with ID (8) and further studies in the Lithuanian population are necessary to confirm this observation. The recognizable microdeletion syndromes were confirmed by FISH in 2.3% of patients. Williams, Di George, Angelman and Prader-Willi syndromes were among the most common. This method was used prior to molecular karyotyping for the confirmation of specific clinical suspicions. According to different studies, known microdeletion syndromes account for 3–9% of ID / DD causes (8).

According to our results, biochemical (metabolic) testing was effective in 4.15% of patients with ID / DD. Inborn errors of metabolism are a group of disorders, caused by the dysfunction of enzyme encoded by a single gene. Screening results revealed by several studies demonstrate that the yield of metabolic testing is 0.2–4.6% (13). This testing is especially effective if suggestive clinical features of metabolic disorders are present in patient's clinical history and physical examination.

Only 0.9% of patients had the monogenic disease confirmed by molecular genetics methods.

Fragile X syndrome was among the most common. The diagnostic yielding of molecular genetics testing in other reported studies account for ~10% of cases (9). The low yielding in our study indicates the need of integration of molecular genetic testing in the diagnostics of ID / DD in Lithuania. The excess of male patients in our study and in many other studies of patients with ID / DD indicates the importance of X linked genes in the developmental processes of brain. Recently exome sequencing has greatly impacted the speed at which new disease genes are identified (14). Exome sequencing will likely be an effective tool for identifying the etiology of many cases of ID / DD.

CONCLUSIONS

According to our results, the yielding of current genetic methods in cases of ID / DD is 40.5%. Currently the most effective are conventional karyotyping and molecular karyotyping techniques, showing that chromosomal alterations (aneuploidies and segmental aneuploidies / amplifications) are the most common cause of ID / DD. Mostly the diagnosis is established in severe cases of ID / DD with congenital anomalies and dysmorphic features. Metabolic testing is especially effective if suggestive clinical features of metabolic disorders are present. The low yielding of molecular genetics methods indicates the need of their integration into diagnostics of ID / DD in Lithuania.

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VAIKŲ SU INTELEKTINE NEGALIA GENETINĖS DIAGNOZĖS NUSTATYMAS: ĮVAIRIŲ GENETINIŲ TYRIMŲ DIAGNOSTINIS EFEKTYVUMAS

Santrauka

Tikslas. Ištirti vaikų, konsultuotų Vilniaus universiteto ligoninės Santariškių klinikų Medicininės genetikos centre, intelektinės negalios etiologijos struktūrą 2009 m. ir įvertinti genetinių tyrimų efektyvumą.

Medžiagos ir metodai. Atliktas retrospektyvinis tyrimas. Išanalizuota 217 ambulatorinių kortelių asmenų iki 18 m. amžiaus, konsultuotų dėl intelektinės negalios / raidos atsilikimo (IN / RA). Surinkti šeimos, asmeninės anamnezės, genealogijos, fizinio ištyrimo radiologinių bei genetinių tyrimų rezultatų duomenys. Palyginta grupė pacientų, kuriems nustatyta genetinė IN / RA diagnozė, su pacientų grupe, kurių IN / RA genetinė diagnozė nežinoma. Analizuota IN / RA etiologijos struktūra, kokie veiksniai turi įtakos genetinei diagnozei išaiškinti, kokie genetiniai tyrimai efektyviausiai nustato diagnozę esant konkrečiai 2009 m. IN / RA etiologijos struktūrai.

Rezultatai. Genetinė diagnozė buvo nustatyta 88 (40,5 %) pacientams. 18 % atvejų genetinė diagnozė buvo nustatyta atlikus standartinį kariotipo tyrimą, 12,4 % – molekulinį kariotipavimą, 4,1 % – biocheminius tyrimus, 2,3 % – FISH, 0,9 % – molekulinius genetinius tyrimus, 2,8 % asmenų fenotipiškai ir remiantis anamnezės duomenimis nustatytas alkoholinis vaisiaus sindromas.

Išvados. Šiuo metu IN / RA genetinė diagnozė efektyviausiai nustatoma standartiniu kariotipavimu ir molekuliniu kariotipavimu. Tai rodo, kad šiuo metu chromosomų aberacijos yra dažniausia žinoma IN / RA priežastis. Dažniausiai genetinė diagnozė nustatoma asmenims su sunkia IN / RA, įgimtais raidos defektais ir mikroanomalijomis. Biocheminiai tyrimai yra efektyvūs, kai kliniškai įtariamas medžiagų apykaitos sutrikimas. Mažas molekulinių genetinių diagnozių nustatymo dažnis rodo efektyvesnių molekulinės genetikos metodų taikymo būtinybę Lietuvoje.

Raktažodžiai: intelektinė negalia, raidos atsilikimas, genetinė diagnozė, etiologija, genetiniai tyrimai