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Pregled
Review

PRACTICAL AND ETHICAL DILEMMAS IN GENETIC TESTING - ILLUSTRATIVE PAEDIATRIC CASES

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Context: genetic testing is a type of medical investigation that identifies changes in chromosomes, genes, or proteins. Different techniques can be employed for these purposes: chromosomal genetic tests, molecular genetic tests, and biochemical genetic tests.

Aims: to analyze and discuss some of the most frequent practical and ethical dilemmas in paediatric genetic testing.

Data sources and extraction: case presentations and literature (PubMed) search.

Results and Conclusions: genomic testing, such as chromosomal microarray and exome sequencing, provide search throughout the genome or large parts of the genome, to indentify chromosomal aberrations and small intragenic mutations, respectively. The complexity of this new approach arises from the fact that genomic testing often results in a discovery of genetic variants of unknown clinical significance. Also, the genomic tests may reveal a predisposition for late-onset disorders such as neurodegenerative or malignant disorders, or to disclose misattributed paternity. Nevertheless, facing different professional and ethical dilemmas seems to be inevitably in the practicing of modern medical genetics, and some of these are addressed in this article.

Descriptors: GENETIC TESTING, GENOMIC TESTING, CHROMOSOME, GENE, ETHICS, PEDIATRICS

Abbreviations:
FISH - fluorescence in situ hybridization

INTRODUCTION

Genetic testing is a type of medical investigation that identifies changes in chromosomes, genes, or proteins. The result of a genetic test can confirm or rule out a suspected genetic condition or help to determine chance of developing or passing on a genetic disorder. Different techniques can be used for genetic testing: (a) molecular genetic tests usually search for intragenic mutations (e.g. Sanger sequencing, next generation sequencing); (b) chromosomal genetic tests analyze whole chromosomes or long parts of DNA to search for aberrations (e.g. karyotyping, fluorescence *in situ* hybridization (FISH), chromosomal microarray); (c) biochemical genetic tests usually study proteins, abnormalities of which could indicate presence of a genetic disorder (1).

Because genetic testing has benefits as well as limitations and risks, it should always be voluntary. Patient or parental decision about whether to be tested is a personal and complex one and should represent their informed choice (2). In countries not having "genetic counselor" as a separate profession, such as Serbia, genetic counseling is a part of integrated genetic service together with diagnostics and management, usually provided by clinical geneticists. In such circumstances, duties of clinical geneticists consists of: (a) interpretation of family and medical histories and physical findings to assess differential diagnosis and the chance of disease occurrence; (b) pretest counseling in the process of offering testing; and (c) post-test counseling to discuss test results, educate about inheritance, management and prevention, and to promote informed choices and adaptation to the risks.

Genomic testing, based on the analysis of whole genome, is the foundation of revolution that is happening today in the field of medical genetics, and tomorrow in other branches of the medicine. The main difference with respect to locus-specific testing (e.g. single gene sequencing) lies in the fact that it is not required to have strong clinical suspicion of a particular disease/syndrome, as well as that simultaneous analysis of a large number or all of the genes/loci can detect causative mutation also in genetically very heterogeneous diseases (1, 3, 4). In the middle of the 20th century the exact number of chromosomes in a human cell was determined, and during the following decades karyotyping was principal genetic test with which it began with, and often ended up testing of the majority of genetic patients. This assay can detect numerical chromosomal

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aberrations, as well as structural aberrations over five megabase in size. Bearing in mind that the incidence of chromosomal abnormalities at birth is around 0.9%, and that prevalence of genetic disorders among children is around 5.3%, it is clear that the majority of genetic disorders in the era of cytogenetics remained undiscovered (2, 5).

In parallel with discoveries of numerous mutations and genetic variants in humans, scientists have provided constant improvements in the field of new technologies and their application in genetic testing. Sanger sequencing allowed analysis of the nucleotide sequence of single gene or part of a gene. It represents the most accurate approach in the search for small intragenic mutations and has been considered as "gold standard"(6). All those who have used this technique to analyze a gene, however, know that it takes much of the time and resources to be performed. Thus, it is clear that this method cannot be successfully applied to the genetically heterogeneous entities, as well as in patients with a less specific phenotype. For this purpose recently we have started to employ technologies which allow simultaneous analysis of a large number of genes/loci, or even analysis of the entire genome (1).

Genome-wide analyses

According to the recommendations of the American College of Medical Genetics, the first step in genetic testing of children with congenital malformations, intellectual disability or autism should be chromosomal microarray (4). This analysis has replaced karyotype which is now indicated in easily recognizable syndromes such as Down syndrome, and in prenatal diagnosis (4). The main advantage of the chromosomal microarray is that, in case of optimal resolution of 10-400 kilobases, it can detect chromosomal aberrations of less than 5 megabase in size throughout the genome, such as microdeletions and microduplications (1). Intragenic mutations typically cannot be detected using this analysis. Further limitation is inability to detect balanced chromosomal rearrangements (4). Recent studies have shown that application of chromosomal microarray in patients with normal karyotype improves detection rate of causative mutations for 15-20% (7).

In case of negative result of chromosomal microarray, it is necessary to reassess patient's phenotype and direct attention to monogenic disorders. If there is a strong clinical suspicion on certain monogenic disorder which is associated with mutation in one or two genes, we would usually use traditional and highly reliable Sanger sequencing (6). However, if literature data indicate that this monogenic disorder can be caused by mutation in one of many genes (e.g. epileptic encephalopathies, cardiomyopathies, congenital myopathies, primary microcephalies), it might be reasonable to consider some applications of next generation sequencing, such as exome sequencing which provides simultaneous analysis of the coding parts of the genome (8). These coding regions represent no more than 1.5% of the genome, but are the seat of 85% of all mutations. Hence, it does not surprise that overall pick up rate of causative mutations is as high as 25-50% for different applications of exome sequencing (9).

Fast and unstoppable introduction of genomic testing into clinical practice changes the basic dogma of clinical genetics - instead of the principle "first phenotyping than genotyping" we have started to adjust to a new principle "first genotyping than phenotyping". The complexity of the new approach arises from the fact that genomic testing often results in a large number of genomic variants detected, of which all but one or a few are of no clinical significance. These "secondary data" could result in a significant patient discomfort; thus, recommendations for their evaluation and reporting have been recently provided (1, 3). Also, the genomic tests may reveal a predisposition for late-onset disorders such as neurodegenerative or malignant disorders, or to disclose misattributed paternity. These challenges make it necessary to proceed with implementation of genome-wide technologies with a caution and appreciation of recommended standards (10, 11). Nevertheless, facing different professional and ethical dilemmas seems to be inevitably, and some of these are addressed in the following case presentations.

CASE REPORTS

Patient 1

An 18-month-old girl was referred to a genetic clinic because of severe growth restriction, pronounced global developmental delay and associated congenital malformations (microcephaly, holoprosencephaly, cleft lip and palate and a number of minor facial dysmorphism). Family history was unremarkable. Analysis of the karyotype showed normal result. Chromosomal microarray disclosed 7q36.3 deletion and 18q23 duplication, which were subsequently confirmed by FISH. Parental FISH testing did not detect rearrangements of chromosomes 7q or 18q in the father, while in the mother it revealed reciprocal translocation between chromosomes 7q and 18q.

Patient 2

A 10-month-old infant was referred to a clinical geneticist because of association of congenital heart defects (atrial septal defect, ventricular septal defect and bicuspid aortic valve) and left-sided oblique facial cleft. Genetic testing was initiated by primary care pediatrician by ordering karyotype, which was reported as normal. We proceeded with chromosomal microarray which revealed atypical heterozygous microdeletion 22q11.21-22q11.23, located between unstable low copy DNA repeat blocks B and G. Further analysis of gene content disclosed that the deletion involves *SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily B, Member 1 (SMARCB1)* gene, a putative tumor suppressor gene frequently associated with malignant rhabdoid tumors. FISH analysis of the parental samples is pending.

Patient 3

A three years and five months old girl was referred to our genetic clinic because of global developmental delay and

mild facial dysmorphism. Karyotype and metabolic investigations were normal. Chromosomal microarray disclosed Xp22.31 duplication. Literature data indicated that phenotype of this duplication only partially resembles the patient's problems. At that time, regardless of being informed about increased recurrence risk, the parents started new pregnancy. Chromosomal microarray performed in parents revealed an identical Xp22.31 duplication in healthy father. Taken together all genetic results, we decided to offer clinical exome sequencing for the girl; it disclosed causative heterozygous *de novo* splicing mutation of the *Euchromatic Histone-Lysine N-Methyltransferase* 1 (EHMT1) gene. By this, the girl was diagnosed with Kleefstra syndrome. We haven't got information about new offspring of the parents so far.

Patient 4

A nine-month-old male infant was referred to our genetic clinic because of suspected pyridoxine-dependent epilepsy. The epilepsy began in newborn period and had beneficial therapeutic response to high doses of pyridoxine. Sequencing of the *Aldehyde Dehydrogenase 7 Family, Member A1* (ALDH7A1) gene showed normal result. After reassessment of the phenotype, we decided to proceed with PNPO gene sequencing; the analysis showed that the patient carries compound heterozygous bialelic *Pyridoxamine 5'-Phosphate Oxidase* (PNPO) gene mutation. In accordance to this result, the diagnosis has been revised and the patient was diagnosed with pyridoxal-phosphate-dependent epilepsy with good therapeutic response to the application of high doses of pyridoxine.

Surprisingly, analysis of parental DNA samples confirmed heterozygous mutation only in the mother and not in the father. Wild type paternal PNPO gene sequence has been confirmed in two separate analyses, excluding by this possible wrong denotation of samples. Still, possible explanations were misattributed paternity, paternal germline mosaicism, or *de novo* mutation of the paternal allele. The parents were informed about these possibilities - first the mother separately, and then the father. They denied misattributed paternity and refused further testing.

Patient 5

A four-year-old girl was referred to the genetic clinic because of profound developmental regression. She started to lose previously acquired skills from the age of one year. At the age of four years she was not able to talk or understand basic commands, and she was severely hypotonic with inability of independent sitting. She has not birth defects. Previously performed karyotype and metabolic assessment showed normal results. We suspected single gene disorder and ordered clinical exome sequencing which demonstrated the presence of homozygous mutation of *Neurexin* 1 (NRXN1) gene located on chromosome 2p. Similar as in Patient 4, we were able to demonstrate heterozygous mutation only in the mother, and not in the father. Further genetic investigation of the family confirmed maternal uniparental disomy in the Patient 5, by which misattributed paternity was excluded.

DISCUSSION

The aim of this article is to describe and discuss some of frequently seen dilemmas in management of pediatric genetic patients.

The case of the Patient 1 addresses two important issues in the daily practice of clinical geneticists. The first question is how far we should proceed with genetic testing before we conclude that the cause of disease is not genetic one, or that it cannot be determined by existing technologies. In many parts of the World karyotyping is still basic diagnostic test for chromosomal analysis. However, if analyzed only by karyotype, we would miss the diagnosis in the Patient 1, and wouldn't be able to inform her parents about high recurrence risk in subsequent pregnancies and options for prenatal diagnosis. In 2013, the American College of Medical Genetics released the statement recommending chromosomal microarray as the first step in genetic testing of individuals having intellectual disability, congenital malformations or autism (4). The second important issue illustrated by this case is presence of two concomitant chromosomal rearrangements in a patient, which should always prompt us to search for underlying balanced translocation in one of parents. In our practice we opt to employ FISH method for parental testing, because chromosomal microarray cannot detect balanced chromosomal aberrations (4).

The case of the patient 2 illustrates advantage of chromosomal microarray in comparison to other methods for microdeletion and microduplication detection, such as FISH. In fact, unlike FISH technique, chromosomal microarray can determine not only presence, but also size and location of a deletion or duplication detected (1, 4). Thus, we were able to diagnose our patient with missing one copy of the SMARCB1 gene. Haploinsufficiency of this tumor suppressor gene produces pronounced susceptibility to malignant rhabdoid tumors; being aware of such predisposition is prerequisite for early diagnosis and prevention.

The case of the patient 3 reflects the typical flow of genetic tests that we order for majority of patients having developmental delay and/or congenital malformations. Chromosomal microarray should be the first step in these patients according to the protocol released in 2013 by American College of Medical Genetics (4). Before referring the Patient 3 to us, the neurologist requested karyotype analysis which was reported as normal; this step is probably not wrong, although it should be noted that aforementioned protocol does not encourage employing karyotype prior to chromosomal microarray. Microduplication discovered on chromosome X was not the likely cause of the Patient 3 problems, which is further reinforced by the confirmation of the same microduplication in her healthy father. Thus, we ordered clinical exome sequencing which can provide simultaneous analysis of dozens or hundreds of genes associated with intellectual disability and developmental delay; EHMT1 gene mutation of de novo origin (parental results negative) was revealed (1). Parents were counseled with slightly increased recurrence risk for ongoing and any further pregnancy. This

risk is equivalent to the likelihood that one parent carries germline mosaicism (around 1-5%).

The case of the patient 4 illustrates the potential problem of misattributed paternity. This possibility shouldn't be overlooked during both pretest and posttest genetic counseling. Literature analysis has shown that there is no unanimous standpoint over how to approach to the couple with suspected misattributed paternity (12, 13). Some authors advocate not disclosing this suspicion to spouses because it is not a medical problem; others, probably majority, believe that it should be discussed only with a woman, while the rest of the authors, probably minority, would disclose the issue to both - men and women (12, 13). Considering this standpoint diversity, it seems justified for a professional to act according to what he or she believes is in best interest of a client. It should be also kept on mind that to some extent, the question of whether parents have a reasonable expectation of disclosure depends on how those expectations were managed in pretest counseling (13). In our genetic clinic at the University Children's Hospital in Belgrade we have incorporated information about potential disclosure of misattributed paternity into what we discuss during pretest counseling, as well as into a written form of informed consent.

The case of the Patient 5 illustrates complexity of genetic testing and counseling. Why didn't we suspect uniparental disomy in Patient 4? This is so because this patient has different mutations on two alels of PNPO gene (compound heterozygosity), and uniparental disomy implies the presence of two identical mutations (homozygosity) on each of two chromosomes because they originate from the same parent. In case of the Patient 5, it would be wrong and disturbing for parents to discuss potential misattributed paternity prior to excluding other possibilities, such as uniparental disomy.

In conclusion, complex genetic testing and unusual results should be managed in collaboration with clinical geneticist or genetic counselor. Careful pretest counseling, including detailed informed consent to be read, discussed and signed, is mandatory. Posttest counseling often requires careful preparation including literature analysis, prior to meeting a patient/family. Growing role of physicians who are not clinical geneticists in a management of genetic patients is welcomed. Chromosomal analysis using array technologies and interpretation of undemanding genetic results should be under their jurisdiction, of course in conjunction with continuous education from the field of medical genetics.

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Sažetak

PRAKTIČNE I ETIČKE DILEME U GENETSKOM TESTIRANJU - PEDIJATRIJSKI PRIKAZNI SLUČAJEVI

G. Čuturilo

Uvod: genetsko testiranje je vrsta medicinskog ispitivanja koja identificira promjene u kromosomima, genima ili proteinima. Mogu se koristiti različite dijagnostičke metode u slijedeće svrhe: kromosomsko genetsko testiranje, molekularno genetsko testiranje i biokemijsko genetsko testiranje.

Ciljevi: ispitati i raspraviti neke od najučestalijih praktičnih i etičkih dilema u pedijatrijskom genetskom testiranju.

Izvor podataka: pretraživanje prikaza slučajeva i literature (PubMed).

Rezultati i zaključci: genomska testiranja, kao što su kromosomski microarray i sekvencioniranje egzona pružaju mogućnost pretraživanja genoma ili velikih dijelova genoma, u svrhu identifikacije kromosomskih aberacija kao i malih unutargenskih mutacija. Složenost ovakvog pristupa proizlazi iz činjenice da genomsko testiranje često rezultira otkrivanjem genetskih varijanti nepoznate kliničke značajnosti. Također, genomsko testiranje može otkriti predispoziciju za razvoj bolesti koje imaju kasni nastup, kao što su neurodegenerativne ili maligne bolesti, ili razotkriti pogrešno pretpostavljeno očinstvo. Štoviše, čini se da je suočavanje sa različitim profesionalnim i etičkim dilemama neizbježno u primjeni suvremene medicinske genetike, a neke od njih su naglašene u ovom članku.

Deskriptori: GENETSKO TESTIRANJE, GENOMSKO TESTIRANJE, KROMOSOM, GEN, ETIKA, PEDIJATRIJA

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