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Editorial

Genetic testing in pediatrics - a narrative essay of challenges and possibilities in Romania

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In spite of extensive medical examinations, many pediatric patients remain without a clear diagnosis. The genetic testing is necessary in the evaluation of some of these challenging cases after a thorough evaluation in the attempt to identify the cause, and for appropriate treatment or genetic counseling when necessary. Newer genetic techniques are more available in pediatric practice due to up-to-date progression and advancement in genetic research and their rapid use in clinical practice.

Genetic investigation enables pediatricians to avoid invasive testing [like muscle biopsies in Duchene muscular dystrophy (DMD) or kidney biopsy in Steroid-resistant nephrotic syndrome (SRNS)]. Genetic testing may help clinicians to avoid harmful therapies (in SRNS cases), to decide the intensity and duration of immunosuppression in SRNS and pre-transplantation therapy, to establish which medications may be contraindicated or are most effective, and also to provide a well-informed genetic counseling to the family (1). Even if some of the genetic techniques are expensive and time-consuming, they are important for appropriate management, prognosis and genetic counseling of the families. For example, in genetic forms the relapse of nephrotic syndrome (NS) kidney transplantation is unusual. In a previous report, the importance of this investigation in late-onset SRNS was proved again (2).

Genetic testing enables identification of the genetic causes of intellectual disabilities in an increased number of children, especially in those cases which associate congenital anomalies and facial dysmorphia. In recent years, multiple studies have clearly demonstrated that molecular genetic tests are recommended instead of cytogenetic analysis, with the exception of those cases with a clinically recognizable aneuploidy syndrome (Down syndrome, Patau syndrome, Edwards syndrome, etc.) or in those patients with a family history of balanced translocations. Patients with intellectual disability (ID) and multiple congenital anomalies (MCAs) may associate copy number variations (CNVs) represented by small losses (deletions, del) and gains (duplications, dup) of genetic material (DNA sample).

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The CNV may be uncovered by chromosomal microarray analysis (CMA), such as comparative genomic hybridization arrays (aCGH) and single nucleotide polymorphism array (aSNP), considered until recently the first-line genetic investigation in ID cases. Certainly, CMA testing has improved the diagnosis in patients with ID. In recent years, the multiplex ligation-dependent probe amplification (MLPA) method has been reported to be a useful, fast and cost-effective test for the evaluation of children with ID (3, 4), especially in ID associated syndromes. Recently, a meta-analysis performed by Srivastava et al. recommended exome sequencing as a first-tier test especially in patients with unexplained neurodevelopmental disorders **(**5**).**

Lysosomal storage diseases (LSD), caused by the deficiency of lysosomal enzymes or non-enzyme proteins, recognize early or late-onset forms. LSDs are known to have a wide spectrum of phenotypes and multiorgan involvement. For these cases, biochemical analyses are important for diagnosis, but genetic analysis is necessary for a precise diagnosis, successful treatment and also for genetic counseling. Many techniques for genetic analysis were used in the LSDs diagnosis: restriction fragment length polymorphism (RFLP), amplification-refractory mutation system (ARMS), real-time polymerase chain reaction, high resolution melting (HRM), multiplex ligation-dependent probe amplification (MLPA), Sanger sequencing and also the newer method next-generation sequencing (NGS) (6). For moment enzyme replacement therapy (ERT) represents the current therapeutic option until gene therapy becomes available. The most frequent LSD is Gaucher Disease (GD). There is a well-known genotype-phenotype correlation in Gaucher disease. A recent study that involved 69 GD cases emphasized the genetic particularities of this disease in Romanian patients and revealed a higher frequency of N370S/L444P compound heterozygotes (35%) and N370S homozygotes (15%) in *GBA* gene compared to Caucasian non-Jews patients (7). The same researchers proved that plasma chitotriosidase is a useful biomarker for treatment response evaluation.

The response to antiepileptic drugs (AEDs) varies between patients with epilepsy (8). Recently, it has been shown that testing for gene variations that might predict adverse reactions (ADRs) and drug response will improve the effectiveness and safety of epilepsy therapies, leading to a precision medicine treatment. Two studies that investigated Romanian children with epilepsy showed that *ABCB1* (T129C, C1236T, and G2677T) gene polymorphisms are not associated with epilepsy and drug responsiveness (9, 10) but an association between *ABCB1* T129C and AEDs concentrations was noticed (10).

Among birth abnormalities, heart defects are the most frequent, with varying severity degrees. Genetics and environmental factors are supposed to be involved in congenital heart defect etiology. When congenital heart defects (CHD) occur together with extra-cardiac anomalies like intellectual disability, developmental delay, dysmorphic face, palatal defects, kidney or ocular abnormalities, etc. they may suggest the presence of a genetic syndrome. Common genetic syndromes associated with different congenital heart disease are Down syndrome and microdeletion syndromes like DiGeorge syndrome (22q11.2 deletion syndrome), Williams syndrome (7q11.23 microdeletion). In the case of microdeletion syndrome fluorescence *in situ* hybridization (FISH) analysis or molecular investigation (MLPA) are necessary. In the case of disorders produced by gene mutation such as Noonan syndrome DNA sequencing analyses are necessary to detect mutations in the *PTPN11*, *RAF1, SOS1, KRAS, NRAS, BRAF, SHOC2* or *MEK1* genes, found in almost 75% of cases. Noonan syndrome (NS), and related syndromes [Costello syndrome (CS), cardiofaciocutaneous syndrome (CFCS), and LEOPARD syndrome]

are caused by mutations in RAS signaling pathway genes. The precise diagnosis in these cases is tremendous as children with „RASopathy" have an increased risk of childhood cancer (leukemia and solid tumors), so they need close follow-up (11). In cases with multiple congenital anomalies such as Emanuel syndrome, the karyotyping (conventional and/ or molecular) represents the first-line of genetic evaluation. If a small supernumerary marker chromosome (sSMC) is detected by conventional karyotyping, the MLPA analysis may represents the choice for a fast and cost-effective method compared to genome array and FISH to establish its origin (3).

Pulmonary arterial hypertension (PAH) is a rare and sometimes lethal complication in CHD. Compared with adults, in children the response to treatment is inferiors and the prognosis is worse. Genetic studies proved the role of genetic factors in the pathogenesis of PAH [bone morphogenetic protein receptor 2 (*BMPR2*) gene; activin A, receptor type II-like 1 (*ACVRL1*)]. Mutations in other genes including T-box4 (*TBX4*), *BMPR1B* and neurogenic locus notch homolog 3 (*NOTCH3*) are involved in pediatric PAH (12). A significantly higher frequency of *TBX4* mutations in pediatric-onset PAH patients was observed, with a 20-year earlier age-of-onset compared with *BMPR2* mutation carriers (13). In a significant percentage of pediatric PAH *"de novo* mutations" were identified by using whole exome sequencing (WES) (13). Other genes were investigated in a Romanian pediatric PAH associated with CHD group, but no association between *ABCB1* polymorphisms (C1236T, G2677T, and C3435T) and evolution was found (14).

Cytogenetic analysis of hematological disorders (like acute lymphoblastic leukemia ALL, acute myeloid leukemia AML, myelodysplastic syndrome MDS, etc.) for identification of chromosomal abnormalities (aneuploidy and chromosomal rearrangements such as deletions, translocations) has become essential for diagnosis, risk-stratification, management of these diseases and monitoring of treatment response (15). Cryptic cytogenetic alterations may be revealed by CMA and by MLPA methods (16). Fusion genes, the result of chromosomal translocation, are important for risk stratification and may be used as prognostic markers in children with ALL (15). Genomic studies in ALL are of clinical importance and are expected to improve diagnosis, monitoring of minimal residual disease (MRD), early detection of relapse, and for implementation of targeted therapy (17). Molecular testing is necessary for children with hematological malignancy. A few studies considered that nucleophosmin 1 (*NPM1)* and transcription factor CCAAT/enhancer-binding protein α (*CEBPA*) mutations defined a particular children AML subgroup with good prognosis and are rare in MDS. The prognostic significance of *FLT3* (fms related tyrosine kinase 3) gene mutation is controversial in childhood AML (17). Taking into account that *NPM1*, *FLT3* somatic mutation are not well-characterized in leukemic children, further studies are needed.

Obesity is a worldwide epidemic problem, with an alarming prevalence increasing especially in our country. It is a multifactorial disease, every factor having an important and additional role in its occurrence: genetic factors, fetal programming, environment, socio-economic status, lifestyle and nutrition, the microbiota of the gut and biomarkers with not always well-identified role (18). Many genes have been investigated to assess their role in obesity. *FTO* (fat mass and obesity-related) is one of the most studied and significantly involved genes linked to obesity. Variants in the *FTO* gene have been proposed to be related to obesity. Other genes proposed to be related to childhood overweight or obesity are melanocortin-4 receptor gene (*MC4R),* peroxisome proliferator-activated receptor gamma (*PPARG),*

angiotensin-converting enzyme gene (*ACE)* (19, 20). *FTO* rs9939609 SNP was significantly associated with obesity in a Romanian pediatric group (19). In another pediatric research II genotype of *ACE* gene was associated with severe obesity while D allele carriers were associated with moderate undernutrition and moderate obesity (20). A recent study reported that *MC4R* rs17782313 and *FTO* rs9939609 have almost no contribution to childhood obesity while *LEPR* rs1137101 and *PPARG-2* rs1801282 may somehow protect against childhood obesity (21).

The genes that encode connexin 26 and 30 *(GJB2*, *GJB6)*, solute carrier family 26, member 4 or pendrin *(SLC26A4 or PDS)*, and otoferlin (*OTOF)* genes are the most frequent genes reported to be associated with non-syndromic congenital hearing loss. Non-syndromic hearing loss accounts for around 70% of all congenital hearing loss. Studies performed on Romanian children with non-syndromic congenital hearing loss have revealed mutations in *GJB2* gene [c.71G>A(p.W24X),c.-23+1G>A,c.299_300delAT, c.313_326del14 (AAGTTCATCAAGGG), c.358_360delGAG (p.delE120), c.551G>C (p.R184P)], and in *GJB6* gene [del (*GJB6*-D13S1854)] (22-24).

With so much information, recent advances in disease understanding, and the broad spectrum of genetic analyses the role of the medical geneticist is crucial to guide us to whom, when, and what kind of genetic testing we should ask for.

The medical geneticist should support pediatricians and family doctors in diagnosis, suggest additional analyses and referrals if necessary, they may provide direct medical care, and give counseling for affected child/ proband and their family.

Conflict of interest

None to declare

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