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THE CHALLENGE IN DIAGNOSIS OF CONGENITAL DISORDERS OF GLYCOSYLATION VERSUS MITOCHONDRIAL DISORDERS: CASE REPORT

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REZUMAT

PROVOCĂRILE DIAGNOSTICULUI DEREGLĂRILOR CONGENITALE ALE GLICOZILĂRII VERSUS MALADII MITOCONDRIALE: CAZ CLNIC

Cuvinte cheie: CDG, IEFT, MD, secvențiere.

Introducere: Dereglările Congenitale ale Glicozilării (CDG) reprezintă un grup de erori înnăscute de metabolism determinate de defecte în sinteza glicanilor și altor glicoconjugate. Maladiile Mitocondriale (MD) apar ca urmare a perturbării căile metabolice localizate în mitocondrii. Atât mitocondiriile cât și procesul de glicozilare sunt aproape în toate celulele din organismul uman. Astfel, ambele grupuri de patologii sunt multisistemice, iar manifestările clinice sunt foarte variate și se suprapun în același timp, ceia ce reprezintă o provocare pentru clinicienii.

Materiale și metode: Se raportează pacientul X, baietel născut fără particularități patologice, dar care din prima lună de viață prezintă manifestări clinice, predominant de afectare a sistemului nervos central cu hipotonie, spasticitate, episoade de opistotonus, convulsii, atrofia nervului optic și trăsături dismorfice.

Rezultate: Luând în considerație tabloul clinic, s-a suspectat o eroare înnăscută de metabolism, inițiindu-se work-upul metabolic. Lactat, amoniac, profilul acilcarnitinic, aminoacizi în sânge și urină fără modificări. S-a determinat episoade de hipoglicemie, hipocalcemie și creșterea transaminazelor. EMG, ECG și RMN cerebral-modificări nepecifice. IEFT – profilul transferinei normal. Cariotip normal. Scorul Clinic după Nijmegen al Criteriilor de Diagnostic al Maladiilor Mitocondriale 5puncte – posibil maladie mitocondrială. Analiza molecular-genetică a fost efectuată utilizând metoda High Resolution Melting (HRM), determinându-se prezența mutației punctiforme m.3243A>G în gena *TL1* al genomului mitocondrial.

Discuții: CDG și MD reprezintă un grup de erori înnăscute de metabolism cu afectare multisistemică, predominant neurologică care necesită un algoritm de diagnostic diferențial bine punctat. Manifestările clinice ale acestor grupuri de patologii de cele mai multe ori se suprapun, îngreunând procesul de diagnostic. Standardul de aur pentru diagnosticul CDG este screeningul prin IEFT, iar pentru maladiile mitocondriale – biopsia musculară. Pasul final în diagnosticul acestor maladii este reprezentat de metodele de secvențiere pe panel de gene sau al întregului exom/genom.

Concluzii: CDG și MD reprezintă o provocare pentru clinicieni, mai ales în stadiul precoce al bolii. Variabilitatea de manifestări clinice conduce spre mimarea patologiilor, astfel acestea fiind subdiagnosticate. Pentru stabilirea diagnosticului cat mai precoce este necesar de a implementa în fiecare departament genetic metodele de screening al CDG și de secvențierea pe panel de gene sau chiar al întregului genom/exom.

SUMMARY

THE CHALLENGE IN DIAGNOSIS OF CONGENITAL DISORDERS OF GLYCOSYLATION VERSUS MITOCHONDRIAL DISORDERS: CASE REPORT

Keywords: CDG, Mds, IEFT, sequencing.

Introduction: Congenital Glycosylation Disorders (CDG) are a group of inborn errors of metabolism caused by defects in the synthesis of glycans and other glycoconjugates. Mitochondrial diseases (MD) occur as a result of disruption of metabolic pathways located in the mitochondria. Both mitochondria and the glycosylation process are present in almost every cell in the human body. Thus, both groups are multisystem afection, and the clinical manifestations are very variable and overlap at the same time, which is a challenge for clinicians.

Materials and methods. Was reported, a boy born at term, who from the first month of life presents clinical manifestations, predominantly affecting the central nervous system with hypotonia, spasticity, episodes of opisthotonus, convulsions, optic nerve atrophy and dysmorphic features.

Results. Considering the clinical picture, an inborn error of metabolism was suspected, initiating the metabolic work-up. Lactate, ammonia, acylcarnitine profile, amino acids in the blood and urine without changes. Episodes of hypoglycemia, hypocalcemia and increased transaminases have been reported. EMG, ECG and brain MRI-nonspecific changes. IEFT - normal transferrin profile. Karyotype was normal. Clinical score by Nijmegen of the Diagnostic Criteria for Mitochondrial Diseases 5 points - possibly mitochondrial disorder. Molecular-genetic analysis was performed using High Resolution Melting (HRM) and revealed the presence of point mutation m.3243A>G in the *TL1* gene of the mitochondrial genome

Discussions: CDG and MD represent a group of inborn errors of metabolism with multisystem involvement, predominantly neurological impairment that require a well-targeted differential diagnostic algorithm. The clinical manifestations of these groups of pathologies often overlap, making the diagnostic process difficult. The gold standard for CDG diagnosis is IEFT screening and for mitochondrial diseases - muscle biopsy. The final step in the diagnosis of these diseases is represented by the methods of sequencing on the gene panel or of the whole exome / genome.

Conclusions. The diagnosis of CDG and MDs is a challenge for clinicians, especially in the early stages of the disease. The variability of clinical manifestations leads to the mimicking of pathologies, so they are often underdiagnosed. In order to establish the diagnosis as early as possible, it is necessary to implement in each genetic department the methods of CDG screening and sequencing on the gene panel or even of the entire genome / exome.

РЕЗЮМЕ

ЗАДАЧА ДИАГНОСТИКИ ВРОЖДЁННЫХ ЗАБОЛЕВАНИЙ ГЛИКОЗИЛИРОВАНИЯ В ОТНОШЕНИИ МИТОХОНДРИАЛЬНЫХ ЗАБОЛЕВАНИЙ: КЛИНИЧЕСКИЙ СЛУЧАЙ

Ключевые слова: Врожденные нарушения гликозилирования, Митохондриальные заболевания, секвенирование, IEFT.

Введение: Врождённые нарушения гликозилирования (CDG) представляют собой группу врождённых нарушений метаболизма, вызванных дефектами синтеза гликанов и других гликоконъюгатов. Митохондриальные заболевания (MDs) возникают в результате нарушения метаболических путей митохондрий. И митохондрии, и процесс гликозилирования присутствуют почти в каждой клетке человеческого тела. Таким образом, обе группы представляют собой мультисистемное поражение, а клинические проявления очень разнообразны и частично совпадают, что является проблемой для клиницистов.

Материалы и методы: Мальчик, рождённый без патологических особенностей, но с клиническими проявлениями с первого месяца жизни, затрагивающими преимущественно центральную нервную систему с гипотонией, спастичностью, эпизодами опистотонуса, судорогами, атрофией зрительного нерва и дисморфизмами.

Результаты: Принимая во внимание клиническую картину, подозревалось врожденное нарушение обмена веществ, что послужило приичиной метаболического обследования. Профиль лактата, аммиака, ацилкарнитина, аминокислоты в крови и моче без изменений. Сообщалось об эпизодах гипогликемии, гипокальциемии и повышении уровня трансаминаз. ЭМГ, ЭКГ и МРТ – неспецифические изменения головного мозга. IEFT - нормальный профиль трансферрина. Нормальный кариотип. Клиническая оценка по Неймегену диагностических критериев митохондриальных заболеваний 5 баллов – возможно, митохондриальная патология. Молекулярно-генетический анализ проводили методом High Resolution Melting (HRM), определяя наличие точечной мутации m.3243A> G в гене TL1 митохондриального генома.

Обсуждение: CDG и MD представляют собой группу врожденных ошибок метаболизма с мультисистемным

вовлечением, преимущественно неврологическими нарушениями, которые требуют целенаправленного алгоритма дифференциальной диагностики. Клинические проявления этих групп патологий часто пересекаются, что затрудняет диагностический процесс. Золотым стандартом диагностики CDG является скрининг IEFT, а при митохондриальных заболеваниях - биопсия мышц. Заключительный шаг в диагностике этих заболеваний представлен методами секвенирования панели генов или всего экзома / генома.

Выводы. Диагностика CDG и MD является проблемой для клиницистов, особенно на ранних стадиях заболевания. Разнообразие клинических проявлений является причиной трудности дифференциальной диагностики, поэтому данные патологии часто недооценивают. Чтобы поставить диагноз как можно раньше, необходимо внедрение во всех генетических отделах методов скрининга и секвенирования CDG панели генов или даже всего генома / экзома.

Introduction. Congenital disorders of glycosylation (CDG) are a group of rare disorders caused by defect in the synthesis of the glycans and in the attachment of glycans to other compounds. The prevalence of CDG is between 0.1- 0.5/100000 populations, 70% is corresponding for PMM2-CDG type, with a frequency of 1: 20,000 in European population. According to the latest research, about 150 types of CDG have been established in over 1350 patients reported with a definitive diagnosis at the molecular-genetic level, with a distribution of 94% cases of CDG type I and 6% - respectively CDG type II. As cellular organs such as the Endoplasmic Reticulum and the Golgi Apparatus are present in virtually all human cells, disruption of glycosylation by affecting the structure of glycoproteins and their functionality is resulting in a greatly varied multisystem impairment (> 80% with neurological damage, 22% - hepatic, 20 cardiac, 10% - immunological, etc.). Thus, many types of CDG are mimicked by other pathologies, which serve as the cause of CDG under diagnosis, with important medical and social values[1,2,3,4]. The disease progress gradually sets in, and the predominant nature of neurological impairment is often confused with Infantile Cerebral Palsy (ICP) and mitochondrial disorders. CDG types have been divides in defect of N-glycosylation, O-glycosylation, lipidlinked glycosylation and combined glycosylation pathways[5]. The most frequent type is the group of N-glycosylation defect, namely the subtype PMM2-CDG, which has more than 700 reported individuals and ALG6-CDG, the second most common subtype. The typical clinical manifestation for PMM2-CDG is hypotonia, strabismus, abnormal fat distribution, inverted nipples, dysmorphic features, feeding difficulties, seizures, proximal muscle weakness and ataxia. The "Golden Standard" in the diagnosis of CDG serves the investigation of blood serum by Isoelectrofocusing of Transferrin (IEFT), implemented in 1984[6]. Other diagnostic methods include mass spectrometry, or matrix assisted laser desorption/ionization time-of-flight (MALDITOF) glycan analysis. The identification of genetic mutations is the confirmatory diagnosis of all CDG[7].

Mitochondrial disorders (MDs) are a group of genetic disorders caused by disturbance in metabolic pathways localized in the mitochondria. The occurrence of this group, is approximately 1 in 8500 individuals. MDs affect many organs, in particular those having a highenergy demand, such as brain, heart, muscle, liver and other [8]. The characteristic features are neurological regression, global developmental delay, muscle hypotonia, muscle weakness, seizures, ataxia, dystonia and spasticity. The gastrointestinal system and growth are almost involved in MDs. The most common phenotype is Kearns-Sayre Syndrome (KSS), mitochondrial encephalopathy, lactic acidemia, stroke-like episodes(MELAS) and MERRF. They have a high percentage of heteroplasmy and the phenotypes of the disease depend this. The biochemical abnormality highly suggestive for mitochondrial disfunctions are elevated lactate levels, increased alanine concentration in blood, and urine organic acids showing elevated levels of Krebs cycle intermediates, 3-methyl-glutaconic acids, ethyl-malonic acid or lactate. Muscle biopsy is a method of MDs diagnosis, but in the last 10 years, the diagnostic approach increased the usage of next-generation sequencing techniques [9].

CDG and MDs are multisystem disorder, that predominantly affect central nervous system and many of their features overlap. For clinicians the diagnosis of this group of inborn errors of metabolism is a challenge, especially in the early stage, that why often are underdiagnosed.

Materials and Methods. We report a boy, first child in a family, gypsy origin (they insist on non-consanguinity), born at term with normal body weight. From the 1st mo of life child manifested pneumonia, after that he became to be irritated with progressive evolution. After that, he developed generalized hypotonia and spasticity of the limbs with episodes of opisthotonus. Periodically patient presented episodes of fever non-correlative with clinical, roentgen and blood manifestations. Other clinical evident findings included dolichocephaly, asymmetry of the face and ear flags, hypotrophy of the distal portions of the lower extremities, minor cutis laxa, congenital stridor, seizures, hepatomegaly, optic atrophy, psiho-motor retardation, congenital crooked legs and diminished osteo-tendinous reflexes. Considering multisystem impairment predominant neurological a metabolic disorder was suspected - CDG or MDs.

A. B.

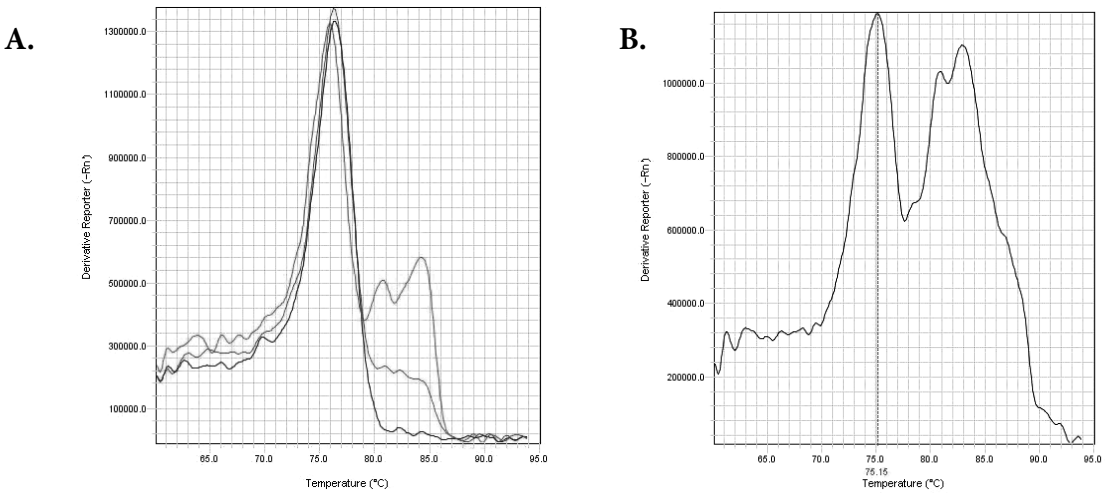

Figure 1. ***a) Melting curve of amplicon-based controls for m.3243A>G mutation. b) Melting curve of the patient's DNA for m.3243A>G mutation***

Results. Considering a suspicion for inborn errors of metabolism was initiated first line metabolic investigations. Laboratory results initially showed episodes of hypoglycemia (2.8-3.0 mmol/l, (ref. val.3.89-5.84mmol/l)), hypocalcemia and increased transaminases, normal lactate, ammonia, amino acids in blood and urine and acylcarnitine profile. ECG showed irregular sinusal rhytm, conduction disturbances in the Hiss fascicle, right ventricular hypertrophy. Second line of investigation revealed on EMG peripheral neuropathy. Cerebral MRI showed hypogenesia of corpus callosum and EEG epileptic activity. To exclude other diseases which may occur with the same clinical manifestations was performed karyotype with normal results (46 XY). Spinal Amyotrophy and Krabbe diseases were excluded. Taking into account the multisystem damage, predominant neurological system and many other features which overlap it was necessary a differential diagnosis between MDs and CDG. For diagnosis of CDG was performed gold standard screening by IEFT in the metabolic laboratory from Mayo Clinic USA under Prof. Morava Eva`s guidance and the result was negative. Considering a normal profile of transferrin on IEFT, was suspected MDs and accumulated score on Nijmegen Clinical Criteria Score for Mitochondrial disease was 5 point - probable MDs. Taking in account that in our country there is no possibility to perform muscle biopsy, was performed genetic analysis using High Resolution Melting (HRM). High resolution melting analysis of amplicons depends on DNA melting in the presence of saturating DNA binding dyes. As the temperature of the solution is increased, the specific sequence of the amplicon (primarily the GC content and the length) determines the melting behavior. When the fluorescence signal is plotted against the temperature, the fluorescence intensity decreases as the double stranded DNA becomes single stranded and the dye is released. The melting temperature (Tm) at which 50% of the DNA is in the double stranded state may be approximated by taking the derivative of the melting curve. The unique pattern of the melting curve, the derivative plot, or the difference plot is used for mutation scanning. Genetic analysis performed using the HRM technique revealed the presence of point mutation m.3243A>G in the *TL1* gene of the mitochondrial genome (fig.1).

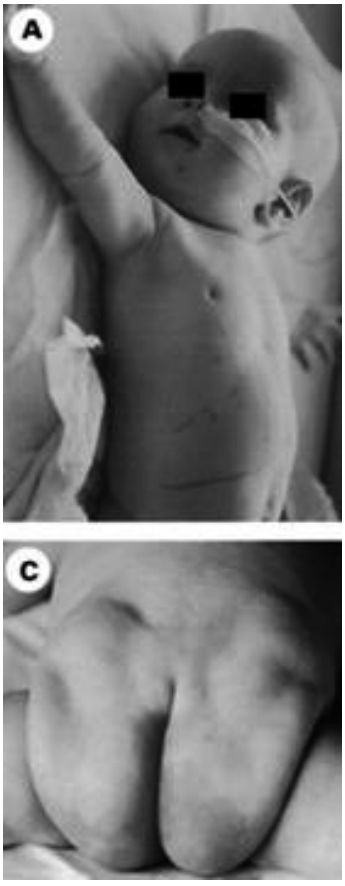

Figure 2. ***A. Inverted nipples. C. Fat pads in CDG***

Dicussions. Congenital disorders of glycosylation (CDG) are a group of rare disorders caused by defect in the synthesis of the glycans and in the attachment of glycans to other compounds. In the last decade with the development of next-generation sequencing methods, the subtypes of CDGs grew rapidly. Considering the presence of glycosylation process everywhere in human body any organ system may be affected, but predominantly the nervous system. The age of onset and severity may range from neonatal lethal to nearly asymptomatic adulthood. The most commonly reported clinical symptoms include developmental delay, failure to thrive, hypotonia, neurologic abnormalities, hypoglycemia, and variable liver, eye, skin, gastrointestinal, immunologic, skeletal, and coagulation abnormalities [10]. A diagnosis of a CDG may be suspected based upon the identification of characteristic symptoms, a detailed patient history and a thorough clinical evaluation. CDG should be considered and ruled out in any unexplained syndrome. The gold method of diagnosis of CDG is screening by IEFT, and the last step is sequencing methods [4].

Neuromuscular manifestations (Maximum of 2 points)	**Central nervous system and other organ involvement (Maximum of 2 points)**	**Metabolic and imaging studies (Maximum of 4 points)**	**Tissue morphology (Maximum of 4 points)**
a. Progressive external ophthalmoplegia (2 points) b. Ptosis (1 point) c. Exercise intolerance (1 point) d. Muscle weakness (1 point) e. Rhabdomyolysis (1 point) f. Abnormal electromyogram (1 point)	g. Isolated central nervous system involvement (1 point) h. Any other isolated organ system (1 point) i. Two or more organ systems (2 points)	j. Elevated blood lactate on three occasions (2 points) k. Elevated cerebrospinal fluid lactate (2 points) l. Elevated blood alanine (2 points) m. Elevated cerebrospinal fluid alanine (2 points) n. Elevated urine tricarboxylic acid (Kreb) cycle intermediates (2 points) o. Elevated urine ethylmalonic, 3-methylglutcaonic, ordicarboxylic acids (1 point) p. Abnormal 31P-MRS (magnetic resonance spectroscopy) inmuscle with reduced Phosphocreatine/Pi ratio (2 points) q. Abnormal T2 signal in basal ganglia on brain MRI (2 points) r. Decreased resting metabolic rate or abnormal exercise studies (cycle ergometry protocol) (2 points)	s. Ragged red fibers on muscle biopsy (2 points if present, 4 points if >2%) t. Diffuse reduction in cytochrome c oxidase histochemical reaction or scattered COX deficient fibres (4 points) u. Strongly succinate dehydrogenase positive vessels by histochemistry (1 point)

Figure 3. ***Nijmegen Clinical Criteria for diagnosis of Mds.***

Mitochondrial diseases(MDs) are the most frequent group of metabolic disorders characterized by dysfunction of functional mitochondria. The prevalence of this group of disorders is about 1 in 4300 cases [11]. The MDs is a multisystem affection with clinical heterogeneity which is most likely due to the heteroplasmy level of mtDNA molecules in cells and the threshold effect: mtDNA molecules are distributed in multiples copies in each cell (polyplasmy) but most pathogenic mutations do not usually affect all mtDNA copies (heteroplasmy) [12]. The most children affected with mitochondrial disease do not have in early stage of disease a classical manifestation which determines the underdiagnosis of MDs. In this group of disorders, the most affected cells are those who require more energy for development such as neurons, skeletal and cardiac muscle, that is why encephalopathy and myopathy are often prominent features in the various type of MDs [13]. Other symptoms as short stature, neurosensory hearing loss, progressive external ophthalmoplegia, axonal neuropathy, diabetes mellitus, and renal tubular acidosis are relevant for respiratory chain dysfunctions [4]. The diagnosis of MDs is based on clinical and biochemical features. An important tool in diagnosis of MDs is Nijmegen Clinical Criteria Score for Mitochondrial disease that (fig. 3) has a high specificity to distinguish between mitochondrial and other multisystem disorders [15]. The CDG may be underdiagnosed when mimic MDs, because both are multisystem disorders with clinical characteristics that may overlap. One of the common features of CDG and MDs who needs a very detailed differential diagnosis is myopathy, which is found almost in all types of diseases. In the most common type of CDG(PMM2-CDG), the myopathy is accompanied with failure to thrive, coagulopathy, mental retardation, hypogonadism and inverted nipples, fat pads which are key sings of CDG disorder suspicion (fig 2). In MDs the myopathy is followed by sensorineural hearing loss, diabetes mellitus, ptosis and progressive external ophthalmoplegia.

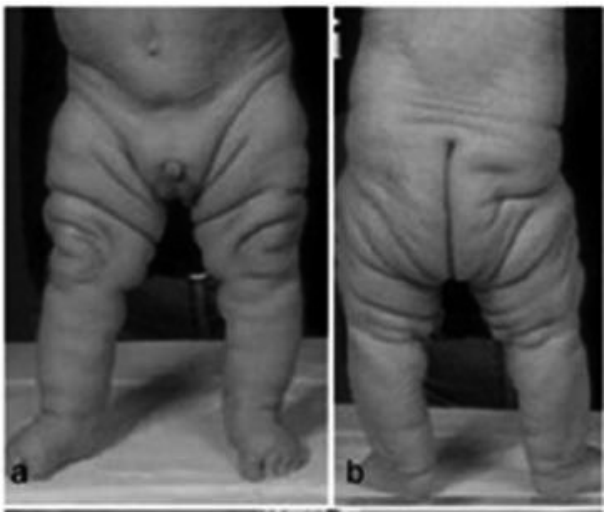

Figure 4. ***Cutis laxa***

Cutis laxa (fig.4) is a condition who first was described as a congenital anomaly syndrome and recently redefined as a CDG. This clinical manifestation can be found in some type of CDG as ATP6V0A2-CDG, COG7-CDG, ATP6V1A-CDG, ATP6V1E1-CDG followed by short stature, joint laxity, muscle weakness, developmental delay, dwarfism, failure to thrive and adducted thumbs [5]. At the same time, the cutis laxa is described in individuals with mitochondrial pathogenic variations PYCR1 and can fully imitate those of ATP6V0A2-CDG including generalized skin involvement, growth and developmental delay, joint dislocations and intellectual

disability. Transferrin isoform analysis (eg, TIEF) is the recommended diagnostic approach to make difference between the two conditions and as the last step, sequence analysis confirms the diagnosis [4]. More than 90% of children develop epilepsy, unspecific clinical features and could fit glycosylation disorders but also with many mitochondrial diseases. In ALG6-CDG the epilepsy is accompanied by ataxia, proximal weakness, developmental delay, cyclic behavioral changes and autistic features. MELAS, MERFF, KSS and MNGIE have been described with very similar phenotype and the lactic acid levels are frequently unreliable, that is why the differential diagnosis is a challenge for clinicians [4]. Liver involvement is characteristic for both groups of inborn errors of metabolism. In mitochondrial disorders hepatopathies mostly present as neonatal acute liver failure or cholestasis, but in CDG one-quarter liver involvement can be progressive and even life-threatening [15]. In CDG was described types with isolated liver affection (MPI-CDG, TMEM-CDG, CCDC115-CDG, ATP6AP1-CDG) and predominant liver impairment in a multisystem affection (ALG1-CDG, ALG3-CDG, ALG8-CDG, COG-CDG, PGM1-CDG) [16,17,18,19]. In some type of CDG, hepatopathies with additional abnormalities as hypercholesterolemia, high alkaline phosphatase levels or decreased ceruloplasmin can facilitate early recognition of CDG. For mitochondrial diseases liver affection is usually progressively associated with neuromuscular symptoms and lactic acidemia. Diagnosis on liver impairment includes screening for CDG by IEFT, lactic acidemia and alanine measurement for MDs, in finally sequencing [20,21,22]. The cardiomyopathy is another symptom that is described in both pathologies, in special in MDs. Hypertrophic cardiomyopathy is reported in almost 20% of patients with mitochondrial affection [23]. Dilated cardiomyopathy with short stature and normal intelligence is described in children with DOLK-CDG and DK1-CDG [24]. In individual with dilated cardiomyopathy and normal intelligence, the clinician should screen for glycosylation abnormalities (IEFT), measure lactate and ammonia, and screen for 3methylglutaconic aciduria by urine organic acid analysis. Congenital cerebellar vermis hypoplasia is the common symptom of CDG, also may be associated with cerebellar and cortical atrophy, clinical manifestations that can be found in MDs in the same time. SRD5A3-CDG present ponto-cerebellar hypoplasia associated with eyes malformations (coloboma, cataract and glaucoma). Several MDs are known with similar phenotype but relatively spread compared with CDG. In individuals with ponto-cerebellar hypoplasia for differential diagnosis should be recommended a detailed eye examination; followed by IEFT; lactate measurements in blood and CSF. In some cases, IEFT can be false negative, therefore gene panel sequencing is recommended. Stroke-like episodes are considered highly suggestive for MDs, however it is reported as a relatively frequent complication in PMM2-CDG. For each disease the path mechanism seems to be different, but the symptoms and MRI picture can be indistinguishable. For establish the diagnosis as quickly and accurately as possible, it is necessary to follow a correct differential diagnostic algorithm [4]. When it suspected a MDs or CDG, it should be initiated first and second line metabolic investigation (acid-base status, electrolytes, glucose, transaminases, ammonia, lactate, amino acids, organic acids, acylcarnitine profile). Then the clinician must make a correlation between a phenotype of patient and lab results and to decide to screen the unclear patient by IEFT for CDG. In dependence of modification of trasferrin profile, will start gene panel sequencing, for last step of diagnosis.

Conclusion. The CDG and MDs are group of inborn errors of metabolism with features that usually overlap. The heterogeneity of clinical manifestations determines misdiagnosis of the disease, that is why need a thorough diagnostic approach. Is necessary to implement a screening methods for CDG and next generation sequencing in each genetic department.

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