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STUDYING INFLUENCE OF MTDNA POLYMORPHISMS AND POLYMORPHIC GENE VARIANTS C677T MTHFR AND A66G MTRR ON CLINICAL MANIFESTATIONS OF MITOCHONDRIAL DYSFUNCTION

Abstract: The research concept of clarifying diagnosis MTHD based on estimates population-genetic characteristics – frequency polymorphisms mtDNA polymorphic variants of genes and enzymes folate cycle. Found variable positions with high volatility and rate of mutations that affect the occurrence of sporadic mutations. It is shown that the population of Ukraine is characterized such distribution of genotypes and allele frequencies of genes MTHFR (C677T, A1298S, G1793A); MTRR (A66G); RFC-1 (G80A), which is characterized by high proportion homozigot MTRR (A66G) mutant allele and 66G, which due to high frequency of lesions of the central nervous system.The character of clinical signs of mtDNA polymorphism carriers – polyorganics, prohrediyentt, clinical polymorphism, genetic heterogeneity of primary delays energothropic bodies. Established separate nosological form MTHD – Leigh syndromes, Leber, Kairns-Seyr, MERRF, MELAS, NARP, confirmed by clinical and genetic, morphological them, biochemical, enzyme, molecular genetic methods. We describe the main clinical features of carriers of polymorphic variants of genes C677T MTHFR and A66G MTRR. These associations MTHD with disabilities re-methylation of methionine as a possible manifestation of altered epigenetic status. Found syntropy phenomenon and proved that the effect on the expression of mtDNA polymorphisms MTHD is due to replace their adaptive role in pathogenic against altered methylation as a major modifier of the genome in violation folate cycle and the presence of certain triggers.

Key words: mitochondrial dysfunction, mtDNA polymorphisms, polymorphic variants of genes of folate cycle, methionine's re-methylation.

Genetics and epygenetics develops quickly and has revolutionary character. New methods of amplification, sequencing give new knowledge every year. It was defined that all genes are fragments of DNA molecule, some of them are recorded in RNA molecule; not all genes encode proteins, some of them have ribosomal and transport RNA as an end product; not all biochemical reactions are controlled by proteins, RNA molecules are as catalyzers; not all proteins are encoded by one gene, several genes take part in their creation; not all 64 codons determine amino acids, three of them mean a translation end; not all DNA fragments are gene parts. There is absurd or egoistical DNA in genome – random sequences of nucleotides or multiple repeats, which are seldom transcriptioned to informational DNA.

Wide human variability, which is caused by quality and quantity changes of chromosomal DNA sequence, is one of the most significant characteristics of human genome variability forms or it can also can be epigenetic phenomena which are determined at molecular level by chemical modification of DNA sequences and chromatin. Hereditary diseases become a leading problem of medicine and mitochondrial dysfunctions (MTChD) are in the lead in scientific inverstigations of a great number of scientists [1, 2, 4, 5, 6, 7, 8, 10].

Multiorgan lesions in MTChD, progreduated and severe clinical course with the final disability, an increasing number of nosologic forms possessing clinical polymorphism and genetic geterogeneity presence of energy metabolic disturbance not only in inherited but also acquired pathological conditions raised the issue about determination mitochondrial pathology. And only intense study of mitochondrial diseases has allowed to generalize this meaning and to define it as MTChD – a typical pathological process, there is no nosologic and etiologic specificity for it and also to consider mitochondrial dysfunction as a new pathobiochemical mechanism of neurodegenerative disorders of a wide range.

The research of this human pathology was transferred to the study of the interaction of gene mutations, the environment, epigenetic factors, nuclear and mitochondrial genomes.

A modern human pathology changes its character, acquires a widespread multiorgan and progreduated course. Y.P. Lisicin untied this phenomenon by the theory of «diseases of civilization and social dysaptation" in 2010, and V. M. Bondar defined this meaning as dizadaptoz – a clinical evidence that the requirements, which environment exposes to a body, don't correspond with genetically established adaptational possibilities of a great part

of population, that is expressed by an increase of a general disease incidence, the frequency of rare hereditary and nonheritable diseases, nosological forms, which hadn't previously met.

S.D. Ellis et al. (2010), E.Y. Grechanina (2011) consider that epigenetic status disadaptoz. Epigenetic processes include multiple genome modifications and first of all a proved process of cytosine bases, which take an active part in gene expressions. Epigenetic modifications are able to fix in a body, influence on a body and genome fate and can be hereditary. An intense development of epigenetic modifications researches has shown that genetics and epigenetics are suitable to each other. Epigenesis is under influence of biochemical reactions and regulates hypo-, hyper – and methylation of cytosine bases. Switching genes on and off, acetylation of chromatine proteins, which are necessary for assembly and packaging of DNA, are also a mechanism of epigenetic status realization.

I.Y.Yurov has recently established, that gene and chromosome instability in brain cells, is one of possible genetic mechanism of autism, schizophrenia, ataxia-telangiectasia, Alzheimer disease manifestation. The main modificator of genome is methylation, which occurs in result of reverse chemical modification of cytosine (C) by joining methyl group with SAM (S – adenosylmethionine) to carbon, and is included in regulation process of gene expression and in support of gene stability.

Methionine plays an especial role in epigenetic regulation, methionine deficiency causes SAM deficiency (SAM is unique donor of methyl groups). Methionine, in turn, is concentrated in mitochondrial membrane and enhance antioxidant characteristics of cells, has overcome evolutionary selection by building antioxidant protein for respiratory chain comlexes. Oxidative stress has given the genetic code of mitochondria.

Participation in these processes of folate cycle as the complex of cascade has been determined, globality of this problem for human health and diseases, but mechanism of folate cycle influence on multifactorial, monogenic (including mitochondrial) and chromosomal pathology is still unknown. It was determined that methyl groups deficit is related with polymorphism of folate cycle because they condition various functional significance of protein products depending on a wide range of biochemical reactions and are considered as risk factors for a great number of diseases.

Triggers, such as a diet, infection, smoking, alcohol and stress, are become important factors, which influence on epigenetic status. The role of triggers in MTChD manifestation still remains unknown.

Mitochondrial genetics is closely associated with epigenetics. It was found out, that histones – high main proteins, which form complexes with DNA, created by nucleosomes and chromatin.

A range of post-translational modifications of histones determines chromatin function, acetylation, methylation, phosphorylation and ubiquitination. In S. Goto's opinion, carbonylation of histones occurs more quickly when a diet is limited and this decreases oxidative stress.

Thus, modern knowledge has combined mitochondrial pathology with a complex system of epigenetic status with participation of methionine metabolism. Therefore studying pathogenic pathways of MTHD formation on basis of genetic surroundings evaluation acquires theoretical and practical meaning.

Bioenergy metabolism disorder becomes more common in human populations, the number of patients with MTHD is being increased and their frequency is 1:3000.

Since multiple mitochondrial proteins are encoded by nuclear genome genes, are synthesized in a cytoplasm and only then transported in mitochondria, MTChD can be a mutation consequence as in the mitochondrial and nuclear genome, which increases a number of mitochondrial pathology and requires differential diagnosis of a modern level [10, 11].

In despite of profound research studies (OI Timchenko, 2011,; CJR Dunning, 2007; M. Lazarov, 2007), populational and ethnical composition restriction of examined families with oxidative phosphorylation pathology (OPH), hasn't been overcome as well as data absence about pathogenic meaning the majority of mutations and about geno – and phenotypic correlations level. These circumstances make fundamental and applied researches significant, which are addressed on understanding mitochondrial pathology in whole.

Mutation specificity determination for certain clinical manifestations, as well as an imagination extension about genetic surroundings influence on gene expression level will help to find out not only pathogenic development mechanisms of energy metabolism disorder, but also to develop adequate methods of prediction, treatment, rehabilitation and prevention of mitochondrial dysfunction.

Studying gene role of polymorphic variants in manifestation of common human disease distribution causes a great interest for the last years [12, 13]. Evidences of a correlation presence of mtDNA variability were found during mtDNA polymorphisms studying, only some works were studying mtDNA polymorphisms associations with neurological lesions. The search work that studied mitochondrial genome variability of Ukrainians has priority among others (working staff: V. Richard, Estonia; T. Shurr, USA, E. Grechanina, Yu. Grechanina, B. Gusar, Ukraine).

Authors supposed possibility of population and genetical background influence on phenotypic manifestations of diseases, that became theoretical basis for study conduction.

Study conception has been developed for

studying influence of mtDNA polymorphisms and polymorphic genes of folate cycle:

Influence of mtDNA polymorphism on mitochondrial dysfunction expression occurs in result of substitution of adaptive role for pathogenic role against the background changed methylation as a main modificator of genome and presence of triggers.

Aim: studying fundamental and applied problems of clinical and genetic variability of mitochondrial dysfunction related with complex interaction of population and genetic features of population, which are able to form predisposition for energy metabolism disorder against the background of changed epigenetic status.

There are following tasks according to the work aim:

1. To determine genetic epidemiology, population and genetic features of mtDNA polymorphisms and polymorphic gene variants of folate cycle enzymes in Ukraine population; To study epidemiology of mtDNA haplotypes in patients with MTChD and clinical and genetic features of patients with mitochondrial dysfunction. Next tasks – To determine the affection range of systems and organs associated with tRNA-leucine and trN-lysine genes and to conduct analysis of clinical MTChD manifestations which are associated with «point" mutations of mtDNA. To study main clinical features of polymorphic gene variants carriers (C667T MTHFR and A66G MTHRR), a character of phenotypic features in patients-carries of polymorphic mtDNA and C667T MTHFR and A66G was next tacks. To develop a continuum of clinical features, an algorithm and genetic navigator for differential diagnostics of MTChD on basis of received data and to determine the effectiveness of a new fetus for differential diagnosis of MTChD was taskc according to the work aim.

Materials and metods. A comprehensive analysis of the following items was performed to achieve the goals and objectives of the thesis study:

– 5895 Registration Cards – database of genetic monitoring (by the rules of work) for ten years;

– 1938 Genetic records of families who have polymorphic gene variants of folate cycle found by selective;

– 200 Registration Cards, of neonates and the results of molecular genetic studies of mtDNA haplogroups;

– 200 Registration Card and the results of molecular genetic studies of polymorphic variants of genes of folate cycle enzymes.

Following patients were examined:

– 652 patients with various forms of hereditary diseases;

– 203 patients with clinically diagnosed MTHD;

– 142 people with no signs of MTHD.

2445 Molecular genetic studies of polymorphisms and 49 point mutations were analyzed.

All examined patients were followed up (dispensary observation) by the thesis defender, who personally carries out the diagnostics, treatment and rehabilitation.

Systematic approach to the problem became the methodological basis of research and this is why, features of mitochondrial DNA polymorphisms as evolutionary formed adaptation factors and polymorphic gene variants of key enzymes of folate cycle were studied at the first stage of the research with help of molecular genetic testing.

Molecular genetic testing was carried out within the limits of two international projects: «Ethnogenomics of Eastern European people: the identification of mtDNA and Y-chromosome haplotypes in Ukrainian population and their analysis by mechanism of mitochondrial diseases expression of Slavs in populations of Eastern Europe", USA.

The detection of DNA point mutations in patients with clinically established by Yu.B. Grechanina diagnosis of MTChD was conducted on a basis of molecular genetics laboratory (Minsk, Belarus, laboratory chief, professor N.G. Danilenko) and molecular genetic laboratory of KhSMGC (laboratory chief V.A. Gusar).

Conducting molecular genetic testing author based on mtDNA haplogroups analysis of 57 patients with suspicion of MTChD and 86 persons without any signs of energy metabolism disorder and 200 neonates.

The second stage – clinical genetic, comprehensive biochemical, cytogenetic, molecular genetic, electronic microscopic (according to indications), morphofunctional (according to indications) testing of patients with clinically established diagnosis of MTChD (203 persons) composed the main group (MG1) including mtDNA polymorphisms carriers (MG2), 91 persons – polymorphic gene variants (C6677T MTHFR, A66G MTRR, mtDNA) carriers (MG3), 75 patients with clinically established MTChD (MG4) (31 with mitochondrial encephalopathy, 3 with MERRF syndrome, 1 with BIDMOAD syndrome, 9 with MELAS, 6 with Leigh syndrome, 12 with MNGIE, 7 with Kearns-Sayre syndrome, 2 with Menkes syndrome, 4 with Leber syndrome) and 142 persons without any signs of mitochondrial dysfunction and folate cycle deficit.

Table 1

Qualitative composition of the studied groups of patients

Patients	Groups – the number of patients, %				
	CG	MG1	MG2	MG3	MG4
Total number	142 (100%)	203 (100%)	37 (100%)	91 (100%)	75 (100%)
men	78 (54,93%)	113 (55,67%)	14 (37,84%)	39 (42,86%)	34(45,33%)
women	64 (45,07%)	90 (44,33%)	23 (62,16%)	52 (57,14%)	41 (54,67%)
at the age up to 11 years	61 (42,96%)	108 (53,20%)	17 (45,94%)	42 (46,15%)	42(56%)
at the age from 12 to 17 years	33 (23,24%)	52 (25,62%)	13 (35,14%)	15 (16,48%)	15(20%)
at the age from 18 to 35	39 (27,46%)	32 (15,76%)	7 (18,92%)	29 (31,87%)	15 (20%)
elder than 35 years	9 (6,34)	11 (5,42%)	0 (0%)	5 (5,5%)	3 (4%)

Molecular genetic testing of polymorphic gene variants C6677T MTHFR, A66G MTRR was carried out for 200 neonates from a population and 1938 patients with various hereditary diseases. Clinical material collection and pheno – and genotypic comparison were made by author in molecular genetic testing. Statistical analysis of received data was carried out by Y.B. Grechanina and A.E. Filatova on the basis of the agreement about scientific technical collaboration with Kharkiv National Polytechnic Institute.

The thesis was carried out at the department of medical genetics of KhNMU, in Ukrainian Institute of clinical genetics of KhNMU and Kharkiv Specialized Medical Genetical Centre on the basis of the agreement of about scientific technical collaboration between KhSMGC, medical genetics department of KhNMU, Novosibirsk Institute of cytology and genetics of Siberian department of RAN, Pennsylvanian University (molecular antropogenetics laboratory, Fhiladelphia, USA).

Molecular genetic testing of blood samples of 200 neonates was carried out in collaboration with Texas University (dermatology and pediatrics departments – B. Brendon B. Holmes, Silvia Guks, Peter L.Rady, Reuben K. Matalon).

Life and disease anamnesis had priority character considering presence of triggers, mediators, progreduated disease course and hereditary character in each individual case.

All method variants of differentional staining were used for studying chromosomes. «C" method was used for identifying some chromosome parts. Constitutive heterochromatin estimation was assessed as highly informative in establishment of epigenetic status disorder.

Results and discussion.

Studying some population genetic markers, which are characteristic for Ukrainian population and studying mtDNA polymorphisms character was being carried out by molecular genetic testing. Estimation of haplotypes frequencies has shown expressed European component presence introduced by corresponding haplogroups (H, U, J, T, V, HV, pre-V, I, W, X, N) summary frequency of which is 95,6% with following distribution: H – 33,5%, U – 20,9%, J – 11,7%, T – 6,7%, V – 5,4%, HV – 3,7%, pre-V – 2,9%, I – 2,1%, W – 2,1, X – 2,5%, N – 1,2%, H, U, J, T (72,8%) haplogroups have the most prevalence. Mongoloid mix (A, B, C, D, Z haplogroups) with a frequency of 2,0% has been found in studying. It has been established 55 polymophic positions with the most variable 16189 and 16204, polymorphisms have been defined in tRNAlys and tRNAleu of encoding region. Polymorphisms in tRNA gene were found in HVS1 , which determine haplogroups 3705G/A (H), 3624 A/G (J), 3594 C/I (12e3), 3336 T/C (Nla), 3552 T/A (C) it has been noted the presence of a wide range of polymorphisms, which characterize T haplogroup (1888 G/A, 8697 G/A, 8860 G, 11251 A/G, 11719 G/A, 11812 A/G, 14766 C/T, 14905 G/A, 15326 A/G, 15452 C/A, 15607 A/G, 15928 G/A) in patients with muscle hypotonior, which is taking its progreduated course. Found mutations in patients with HVS1 types of mtDNA, which determine haplogroups H and X, have allowed us to confirm genetic background influence as the one of additional mechanism of mutational process (V.A. Gusar).

Estimation of mtDNA haplotypes frequencies in 57 patients with clinically established diagnosis of mitochondrial pathology, 86 persons from control group and 200 persons from Ukrainian population (three generations) has showed Eurospecific haplogroups of mtDNA: H, pre-V, V, J, T, U, I, W, X, N, the frequences of which are 24,0%, 2,0%, 12,0%, 16,0%, 18,0%, 2,0%, 2,0% and 8,0%, respectively, total frequence is 84,0%. Asian haplogroups C, A, were found with the frequency of 4,0%. High frequence of T (16,0%), U (14,0%), X (8,0%), N

(10,0%) haplogroups in comparison with controls was likely conditioned by instable positions 16189 and 16294, contained in the main nucleotide motives of these haplogroups.

Interactions between genome and epigenome have increased a number of disease causes, which can be hereditary occur de novo, be genetic or epigenetic. Their appearance depends on an environment, which can change epigenome (also DNA methylation) and it influences on a frequence of neurological, psychic oncologic diseases.

Molecular genetic testing has been carried out and genetic epidemiology of polymorphic gene variants of folate cycle enzymes in 200 neonates (from population) has been studied because the one from biological markers of changed epigenetic status is DNA methylation connected with folate cycle function. Genotypes and allele frequencies of MTHFR C677T, A1298C, G1793A, Mtrr A66G, RFC-1 G80A were studied. Received data show that there is lower prevalence in individuals, who are homozygotes for gene C677T MTHFR (7,04%) and higher in Ukraine who are heterozygotes (40.70%) in comparison with other populations (table 2) (fig.1)

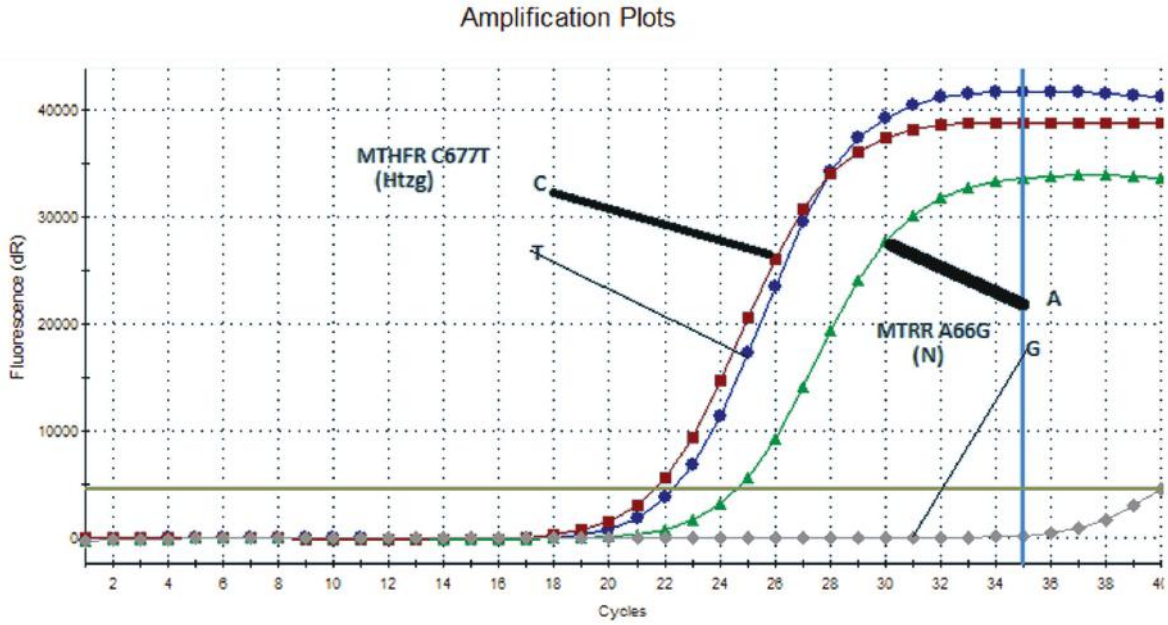


Fig. 1. Reasearch results of polymorphic variants of genes MRHFR C677T (Htzg) and MTRR A66G by PCR method in real time

Gomozygote prevalence is the highest in Ukraine (35,5%) as well as allele frequence of MTRR A66G (57,0%). Gomozygosity for RFC-1 G80A (CG) allele was 38,42% and was higher in controls, allele frequence of RFC-1 G80A for Ukrainian population was lower (38,4%).

Studying heterozygotes distribution by receive new data. It is known that interactions between two and more genes, which encode proteins, taken participation in homocysteine metabolism, form negative effects of polymorphism.

Structure distribution of heterozygotes, two, three, four and five alleles of MTHFR, MTRR, RFC-1 were registrated and all possible combinations of complex heterozygosity were found. Double homozygosity for MTHFR C677T/MTRR A66G and MTHFR C677T/RFC-1 G80A (GG) had the frequence of 3,5 and 2,1%, respectively. Double homozygosity for MTRR A66G and RFC-1 G80A (GG) heterozygosity for polymorphic sites MTHFR C6771/A298C had the frequence of 12,1%.

Table 2

The distribution of genotypes and allelic frequencies of MTHFR (C677T, A1298C, G1793A) MTRR (A66G) RFC-1 (G80A) genes

Polymorphisms	Ukrainians	Ashkenazi Jews *	Afro-american s*	Europeans*	Spaniards *
MTHFR C677T	N=199	n=155	N = 97	N=159	n = 96
Homozygotes	7.04% (n = 14)	26.5% (n = 41)	1.0% (n=1)	11.3%(n = 18)	26.0% (n = 25)
Heterozygotes	40.70% (n = 81)	42.6% (n = 66)	21.6% (n = 21)	42.8% (n = 68)	43.8% (n = 42)
Norrn	52.26% (n = 104)	31.0%(n = 48)	77.3% (n = 75)	45.9% (n = 73)	30.2% (n = 29)
Allelic frequence	27.39% (109/398)	47.7% (148/310)	11.9%(n= 23/194)	32.7% (104/318)	47.9% (92/192)
MTHFR A1298C	N = 200	n=149	N = 97	N=159	n = 96
Homozygotes	8.50% (n= 17)	8.1% (n=12)	2.1% (n = 2)	8.8%(n=14)	4.2% (n = 4)
Heterozygotes	39.50% (n = 79)	38.3% (n = 57)	26.8% (n = 26)	47.2% (n = 75)	27.1% (n = 26)
Norrn	52.00% (n = 104)	53.7% (n= 80)	71.1% (n = 69)	44.0% (n = 70)	68.8% (n = 66)
Allelic frequence	28.25% (113/400)	27.2% (81/298)	15.5% (30/194)	32.4% (103/318)	17.7% (34/192)
MTHFR G1793A	N=195	n=117	N = 97	N=159	n = 95
Homozygotes	0.00% (n = 0)	0.0% (n = 0)	0.0% (n = 0)	0.6% (n=1)	0.0% (n = 0)
Heterozygotes	4.62% (n = 9)	2.6% (n = 3)	6.2% (n = 6)	12.6% (n = 20)	11.6%(n =11)
Norrn	95.38% (n = 186)	97.4% (n = 114)	93.8% (n = 91)	86.8% (n = 138)	88.4% (n = 84)
Частота алелля	2.31% (9/390)	1.3% (3/234)	3.1% (6/194)	6.9% (22/318)	5.8% (11/190)
MTRR A66G	N = 200	n=123	N = 97	N=159	n = 96
Homozygotes	35.50% (n = 71)	19.5% (n = 24)	10.3% (n= 10)	29.6% (n = 47)	7.3% (n = 7)
Heterozygotes	43.00% (n = 86)	47.2% (n = 58)	47.4% (n = 46)	49.7% (n = 79)	42.7% (n = 41)
Norrn	21.50% (n = 43)	33.3% (n = 41)	42.3% (n = 41)	20.8% (n = 33)	50.0% (n = 48)
Allelic frequence	57.00% (228/400)	43.1% (106/246)	34.0% (66/194)	54.4% (173/318)	28.6% (55/192)
RFC-1 G80A	N=190	n=122	N-101	N=131	n=108
Homozygotes	38.42% (n = 73)	28.7% (n = 35)	20.8% (n = 21)	29.0% (n = 38)	26.0% (n = 30)
Heterozygotes	43.16% (n = 82)	45.9% (n = 56)	45.5% (n = 46)	47.3% (n = 62)	43.8% (n = 54)
Norrn	18.42% (n = 35)	25.4% (n = 31)	33.7% (n = 34)	23.7% (n = 31)	30.2% (n = 24)
Allelic frequence	40.00% (152/380)	48.4% (118/244)	56.4% (114/202)	47.3% (124/262)	47.2% (102/216)

In this analysis: 7,0% of Ukrainian population (n=199) were homozygous for MTHFR, 35,5% were homozygous for RFC-1 G80A. Moreover, 3,5% (n=199) and 3,2% (n=190) had population had homozygosity for MTHFR C677T/MTRR A66G and MTHFR A1298C/RFC-1, respectively, 12,6 (23/190) – heterozygosity for polymorphic sites of MTHFR C677T/A298C.

Received data analysis has highlighted that Ukrainian population has a high deficit of folate and, accordingly, a high risk of nervous system affection, that is confirmed by genetic monitoring results (fig. 1).

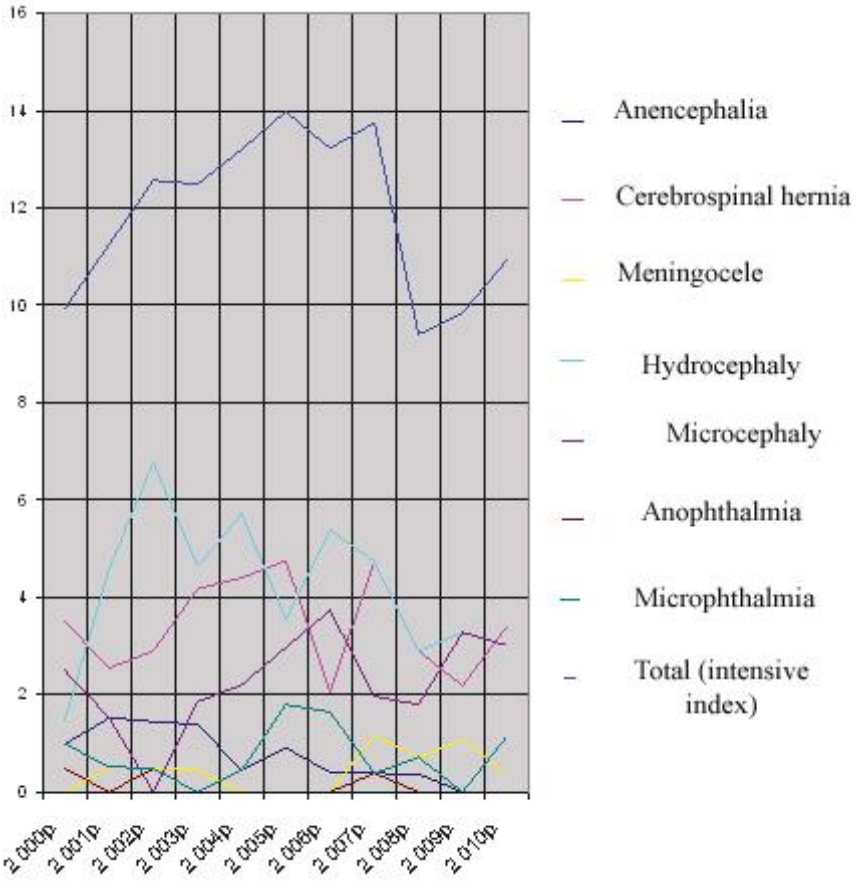


Fig. 2. Comparative characteristics of the CDF of nervous system according to genetic monitoring (2000-2010) (intensive index)

The key enzyme of folate cycle is MTHFR enzyme. The frequence of its heterozygous allele of 677CT is 43,3% (in studied population) that means enzyme activity decrease (by 35%) in great number of examined patients. Gomozygous allele of 677CT is in 8,7%, that decreases enzyme activity (by 70%) in a great number of people. Considering that this enzyme is closely connected with energy metabolism because takes part in CoQ synthesis, it is evident that there is the influence of this polymorphism on mitochondrial function in norm and in pathology.

It is reasonable to agree with the supposition, that allele carriers of 677T has selective advantage in natural selection because activity decrease of MTHFR during a hunger was a reason of remethylation decrease of homocysteine, and therefore tetrahydrofolate is available for DNA and RNA synthesis. Therefore our ancestors could survive and transfer this feature to following generations.

MTRR A66G polymorphism, prevalent in other populations, has appeared with a high allele frequence among examined patients. There were 35,5 % of homozygote carriers of A66G in Ukrainian population (in Europe – 29.6%). There were 37.0 % of homozygote carriers and allele frequence was 58.0 % in patients. It means that such population quantity has a risk of nervous system affection independently of folic acid intake because we discuss the disorder of cobalamin biogenesis.

It has been shown that polymorphisms combination of C677T MTHFR and A66G MTRR can manifest in other way than total effects each of them. Their common influence was a cause of the amplification or the compensation of phenotypic manifestation, therefore, influenced on survival of people with various compound genotypes. More likely that it depends on other components of genome function – on triggers and mediators.

Received data confirm influence possibility of polymorphic gene variants MTHFR on clinical manifestation character of MTChD: level change of S-adenosylmethionine involves energy metabolism in pathologic process.

Phenotypic estimation of data of 203 patients (MG1 (the main group1) and CG9 control group)) has been made/ Homogeneity analysis of sex and age groups was carried out using X2 criterion: group division on sex and age corresponded to evidence requirements on the basis of these criteria. Figure 4 shows the difference of phenotypic features in MG1 and CG; lesions of nervous, urinary system and muscles were prevalent in examined patients with MTChD (Kramer's coefficients: 037.040 and 0.48, respectively).

Phenotype analysis of patients with MTChD has shown the most significant changes in muscle and nervous system. Mitochondrial pathology is characterized by muscular system affection, because this system is the most highly energotrophny organ. Various degree of muscular hypotonia was being clinically shown and in some cases – distonia was followed by muscular weakness, increased fatiguability, diffuse muscular pain, muscular atrophy and hypotrophy. Such changes in MG1 were found in 103(60.10%) patients, while in CG – only 17 (11.97%) persons had mild muscular affection. Found changes has been confirmed by statistical calculation and show the difference of phenotypic signs between the main and control groups (table 3, 4). These clinical signs were morphologically characterized by symptoms of «red torn fibers» in polarographic research, in such cases there was enzymatic activity decrease, in electronic microscopy – structural and quantitative changes of mitochondria. Profound studying central nervous system condition in examined patients has allowed us to find a wide range of changes, which shows, on the one hand, certain specificity of mitochondrial dysfunction.

Table 3

Contingency table by the sign of $x_0^{1,5}$ «muscles »

Signs of gradation		Group		□	$\chi^2_{кр}$	*V*
		MG1	CG			
$x_0^{1,5}$	0	81 (39,9%)	125 (88,03%)	206 (127,93%)	81,80	0,49
	1	103 (50,74%)	17 (11,97%)	120 (62,71%)		
	2	19 (9,36%)	0 (0%)	19 (9,36%)		
□		203 (100%)	142 (100%)	345 (200%)		

Table 4

Contingency table by the sign of $x_0^{2,1}$ «nervous system»

Signs of gradation		Group		□	$\chi^2_{кр}$	V
		MG1	CG			
$x_0^{2,1}$	0	68 (33,5%)	92 (64,79%)	160 (98,29%)	49,62	0,38
	1	33 (16,26%)	30 (21,13%)	63 (37,39%)		
	2	102 (50,24%)	20 (14,08%)	122 (64,32%)		
□		203 (100%)	142 (100%)	345 (200%)		

Generalized disorder of digestion dysfunction is neurogastrointestinal encephalopathy. Intestinal symptoms initiations occurred in childhood or postpubertal period and manifested by chronic diarrhea, stasis, sickness, vomiting, that was a cause of exhaustion and cachexia. J. Shoffner's data (2010) show the loss of longitudinal layer of muscular tunic, formations and disruptions of diverticule, intestinal sclerodermia and pseudoimpenetrability. Electrophysiological researches have revealed lesions of CNS and inner organs together with cardiac penetrability, lactate-acidosis.

Extraintestinal symptoms were characteristical for MTChD. J.Finsterer (2010) confirms this fact. Grogth delay, leucodystrophy and ataxia in brain, ophthalmoplegia, ptosis, neurosensory deafness were noted. Craniocerebral nerves were involved in this process (dysartria, dysphonia, prosopoplegia) cardiac blockage was often developed. Patients had exercise intolerance weakness and «torn red fibers" found in muscular biopsy. MNGIE syndrome course (12 patients) has changed from progreduated to longterm remission in treatment process.

Sceletal changes were phenotypic signs of MTChD. A chest was changed in 109 patients (MG, 53,69%) and 46 persons (CG, 32,39%). These data show that muscular frame weakness and secondary connective tissue dysplasia (figures 3a, b, c) become causes of sceletal disorders (tables 5, 6).

Table 5

Contingency table by the sign of «chest”

Gradation of signs		Group		□	$\chi^2_{кр}$	V
		MG1	CG			
$x_0^{1,17}$	0	94 (46,31%)	96 (67,61%)	190 (113,92%)	15,44	0,212
	1	70 (34,48%)	31 (21,83%)	101 (56,31%)		
	2	39 (19,21%)	15 (10,56%)	54 (29,77%)		
□		203 (100%)	142 (100%)	345 (200%)		

Table 6

Contingency table by the sign of $x_0^{1,18}$ «spine”

Gradation of signs		Group		□	$\chi^2_{кр}$	V
		MG1	CG			
$x_0^{1,18}$	0	78 (38,42%)	82 (57,75%)	160 (96,17%)	12,75	0,192
	1	20 (9,85%)	8 (5,63%)	28 (15,48%)		
	2	105 (51,73%)	52 (36,62%)	157 (88,35%)		
□		203 (100%)	142 (100%)	345 (200%)		

Differential molecular genetic diagnostics of MTChD, encoded by «point” mutations, was carried out in 49 patients from 75 patients of the main group (MG1), which have certain nosologic forms. Mutation search (T8993G) in Leigh and NARP syndromes was in ATP6, TRNL1 snp, SURF1, ND5snp 12706 gene (n=9).SURF1 (1 patient) and ND5snp 12706 (1 patient) mutation were found. In de novo mutation (12706) such polymorphisms were also found: a new mutatuion (tRNA-leucine) 3624 A/G, AC substitution (tRNA-leucine) syn; polymorhisms (tRNA-lysine) 8860 G, AC substitution (tRNA-lysine) trh/ala, a new mutation (tRNA-lysine) 9018 T/C, AC substitution (tRNA-leucine) syn (1 patient).

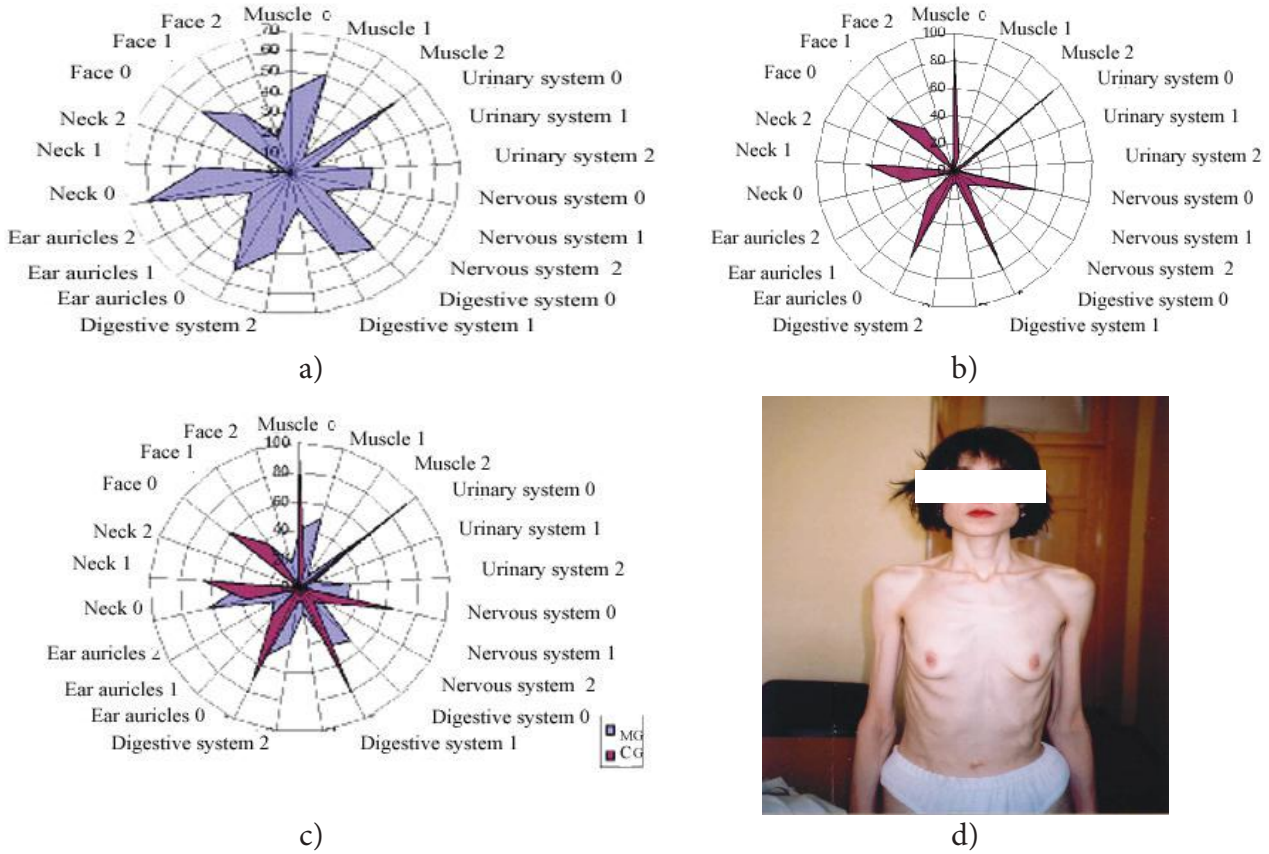


Fig. 3. (a) – The diagram of MG; (b) The diagram of CG;(c) the diagrammms of the distribution of phenotypic traits (muscle, urinary system, nervous system, digestive system, ear auricles, neck, face) of the compared groups of MG and CG: (c) the diagrams of MG and CG; (d) – MNGIE syndrome (Mitochondrial neurogastrointestinal encephalopathy)

Mutation search (A32434) was being carried out in MELAS syndrome in tRNAleu gene (n=27). Mutations were not found, but polymorhisms were found. Large fragment deletion of mtDNA (n=6) was found in 3 patients with Kearns-Sayre syndrome. There are no mutations in other cases, that is explained by often search of mutations.

Carried out within the limits of common project with collaborators from USA and Russia study of expression of mitochondrial diseases in Slavonic population of Eastern Ukraine has shown search difficulty of mitochondrial mutations, besides, various nuclear gene defects and mtDNA mutations occur in the majority of mitochondrial syndromes. Only collaborative study has allowed to find, confirm and describe mtDNA mutation (for the first time), which was in ND5 gene ("hot point"). Such mutation was being considered as unique mutation because its association with phenotype of Leigh syndrome hadn't been confirmed. Hypothesis confirmation was found by a. Morgan Hudless J.A., Hanna M.G., (2009):

Leigh syndrome appearance is followed by additional mechanisms, which define phenotypic expression, such as the genetic background and ecological factor.

This hypothesis has been confirmed by differential diagnostics of MTChD: the patient with suspicion of mitochondrial encephalopathy has heteroplasmic mutation (12706C ND5), which is associated with clinical manifestation of fatal Leigh syndrome with unusual brain lesion and is a new one, developed in mother's (fig. 6).

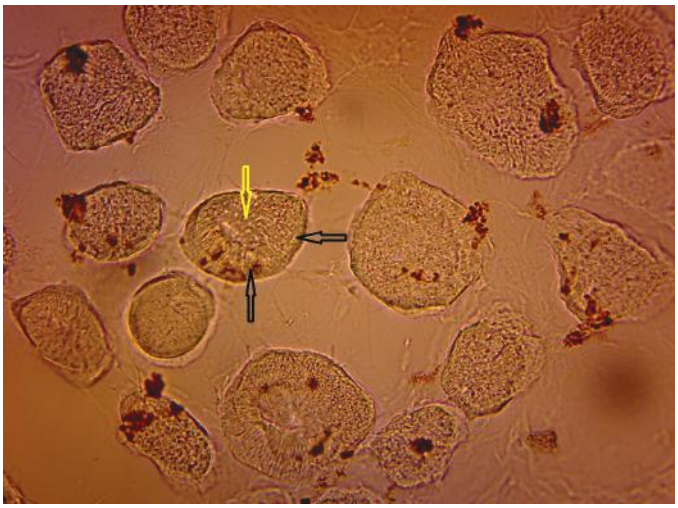


Fig. 4. Morphological phenomenon of «ring-shaped fibers» (ring or ringed fibers).
Because the staining reveals the activity of mitochondrial enzymes, the ring shows the anomalous distribution of mitochondria in the fiber (cytochrome 40s ed). The yellow arrow points to the center of the fiber, which is different from the color around the fiber (black arrows).

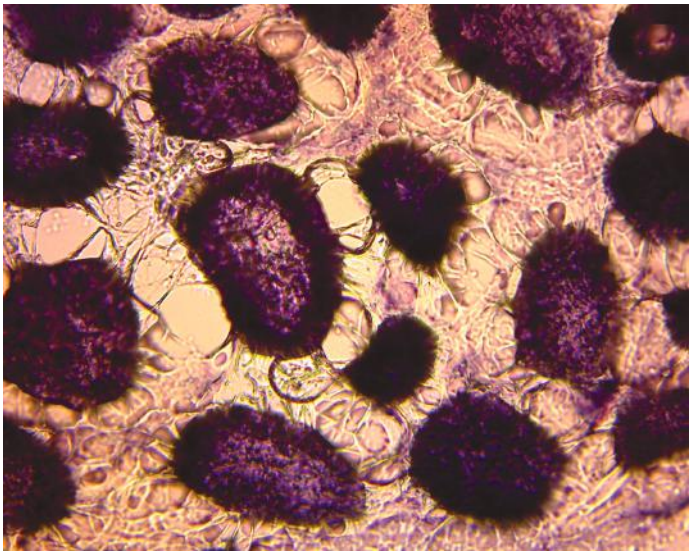


Fig. 5. NAD40 – ring-shaped fiber in the center.
The phenomenon of fiber type disproportion – the tiny fibers are colored more intensively than the big fibers (they have the high activity or the number of mitochondria and enzyme)

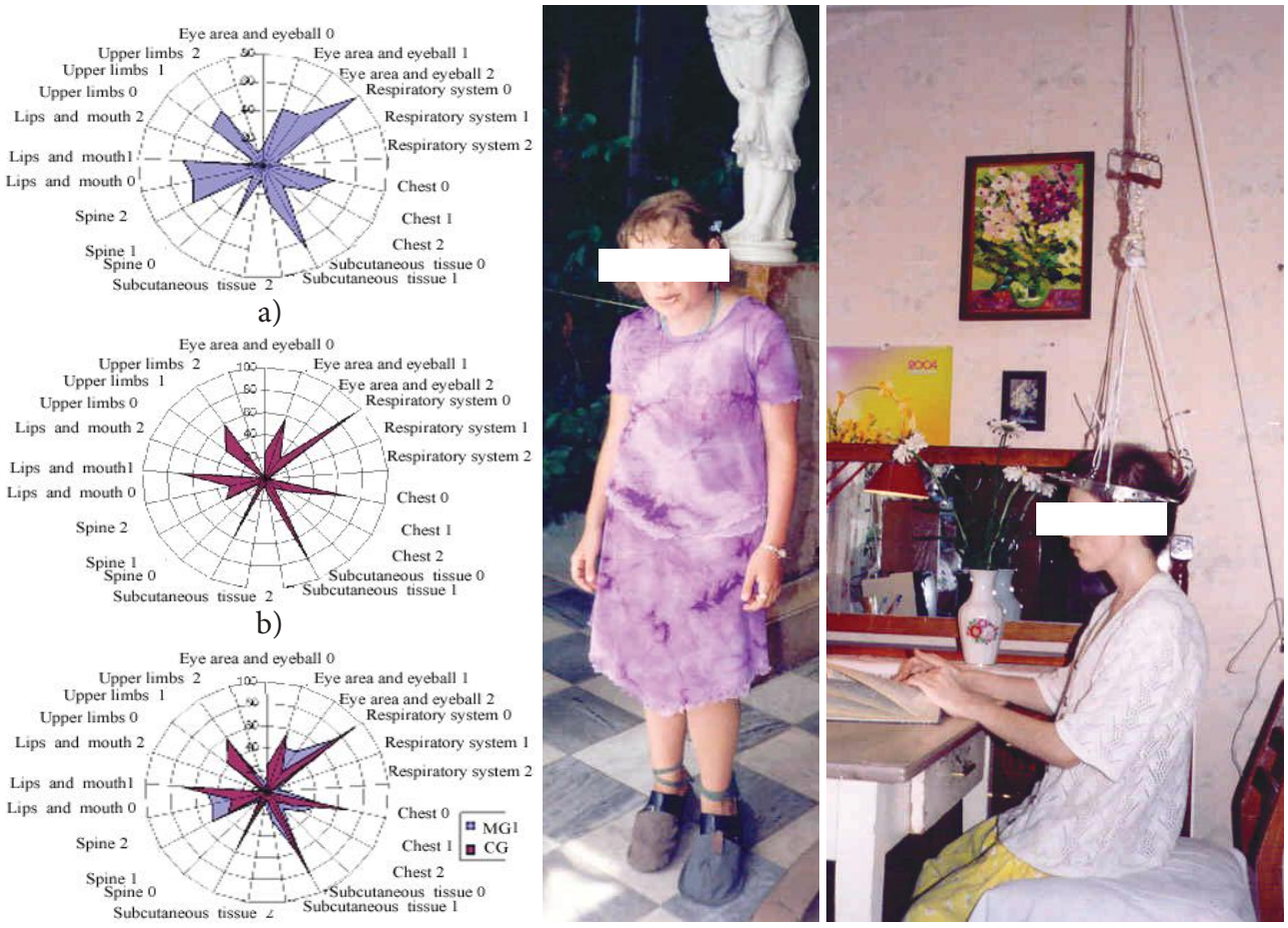


Fig. 6 .The diagrams of the distribution of phenotypic traits (eye area and the eyeball, respiratory system, upper and lower jaws, chest, subcutaneous tissue, the spine, lips and mouth) of the compared groups of MG and CG: (a) the diagram of MG1; (b) the diagram of CG; (c) the diagrams of MG and CG; (d) the patient with geno-and phenotypic syntropy: Turner's Syndrome, a mosaic form 46HH / 45H. Mucopolysaccharidosis. MTHD. homocystinuria (type III) mtDNA polymorphism 8697G / A, 8860 G in the tRNA-lysine, and the C677T MTNFR Htrzgt A66 MTRR Hmzgt

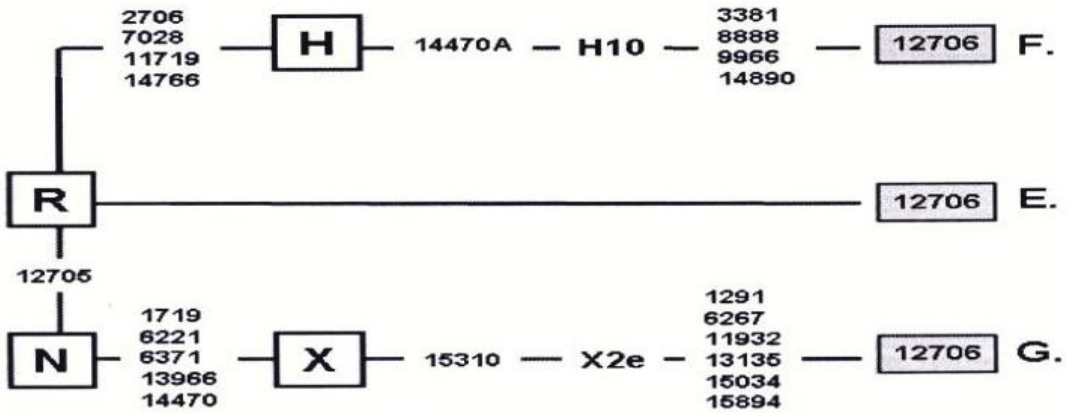


Fig. 7. Phylogenetic relationships among the different haplotypes with the mutation 12706S. To build the network we used only sequences that encode. MtDNA haplotypes of F., G., E. probands are presented with12706S mutation. The main philogenetic areas of these haplotypes of mtDNA are indicated in the squares.

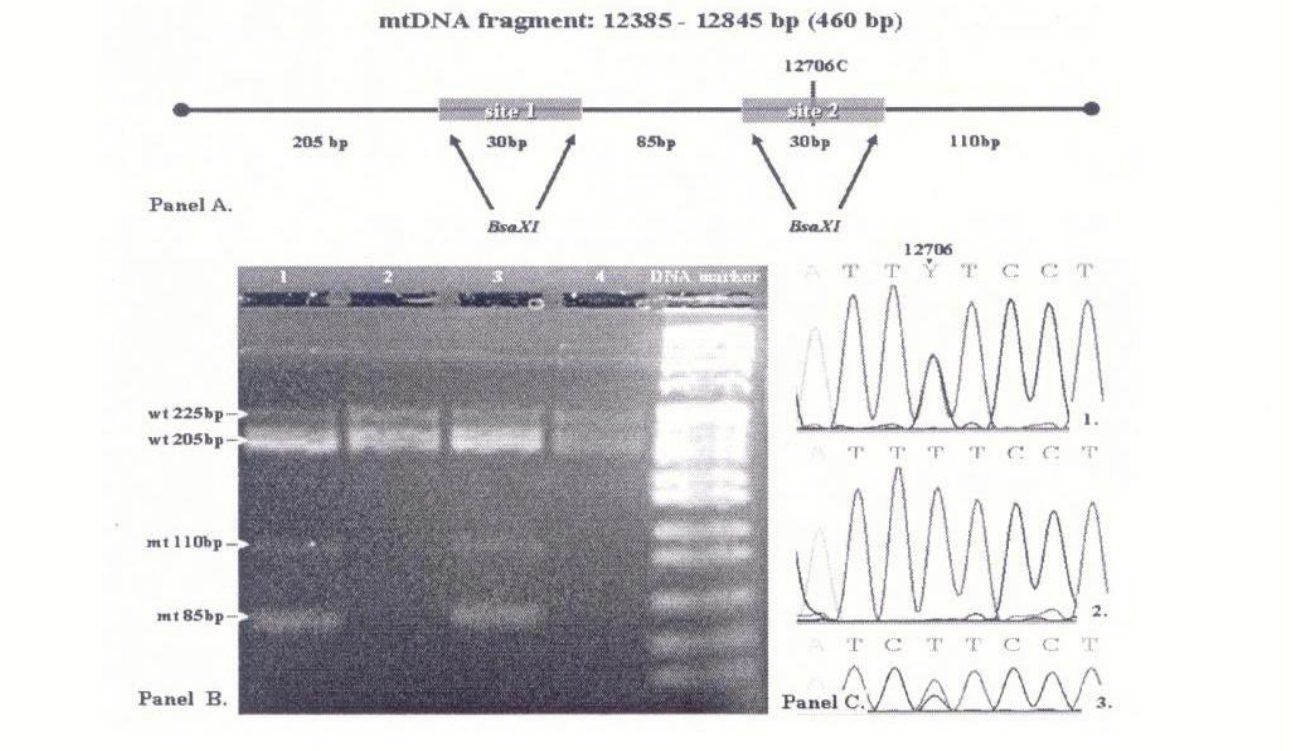


Fig. 8. Schematic overview of the method of PCR-RFLP (PCR-RFLP) to identify mutation 12706S. Panel A. The diagram of amplified fragment of mtDNA, indicating restriction site BsaXI. The second restriction site BsaXI is missing in the wild type of mtDNA, which leads to the distribution into 3 fragments of 225, 205, and 3O bps. In the presence of mutation 12706S, BsaXI restriction site is divided into five fragments of 205, 110, 85, 3O and 3O bps. Panel B. The image of amplified fragment of ND5 gene with BsaXI restriction in the gel. Probands G. and F. (lines 1 and 3) contain a combination of molecules of the mutant and wild types, while the mother of the proband G. (line 2), as well as in the negative control (line 4), has only the bands of wild-type with the absence of mutation. Panel C. Electrophoregram of the proband G.(1), his mother (2) and the proband F. (3), with numbered lines, which corresponds to the panel B.

Carried out phylogenetic analysis of positive cases with mutation 12706C has shown that all mutations have different haplogroups of mtDNA (these mutations occur by independent mutational events). This mutation aspect (12706C) has confirmed its pathological meaning in syndrome development.

Parametric studies were carried out to confirm pheno – and genotypic correlation in such case, their conduction was in collaboration with several world molecular genetic laboratories. mtDNA was being analyzed by southern-blotting hybridization with consequent restriction for mutation exclusion (mtDNA reconstructions, which often occur). mtDNA – encoding region was amplificated with specific kit of primers. Samples of proband and mother were analyzed by sequencer ABJ 3100 Gene Analyzers in Sequencing Centre of Genetics Department of Pennsylvanian University. Mutation presence (12706C) was confirmed by using mutationally specific restriction, besides screening for ND5 mutation was carried out among 187 healthy people and patients with MTChD, which suited by haplogroup chosen participants from other biomedical projects (S.I. Gadanov, T.Shurr).

Sequencing of mtDNA-encoding region of proband G. has revealed 24 main nucleotide substitutions J. mtDNA had polymorphic sites which are characteristic for haplotypes X 2c. This mtDNA line origins from South Siberian and often occurs in European population (Lebon S. 2003). Mutationally specific analysis (PCR-REJP) of 127063 mutation and sequencing of mitochondrial genome have show that this mutation is in heteroplasmic condition (mutative threshold is 50%) in proband, but is absent in blood of proband's mother. These data supported a hypothesis of the most possible appearance of de novo 12706C mutation in fetus cells of proband's mother.

Studying mtDNA polymorphisms has shown a possibility of their pathogenetic action, influence on signs manifestation of hereditary pathology. Clinical features of patients with signs of energy metabolism disorder were studies in order to define a probability of such influence on MTChD manifestation.

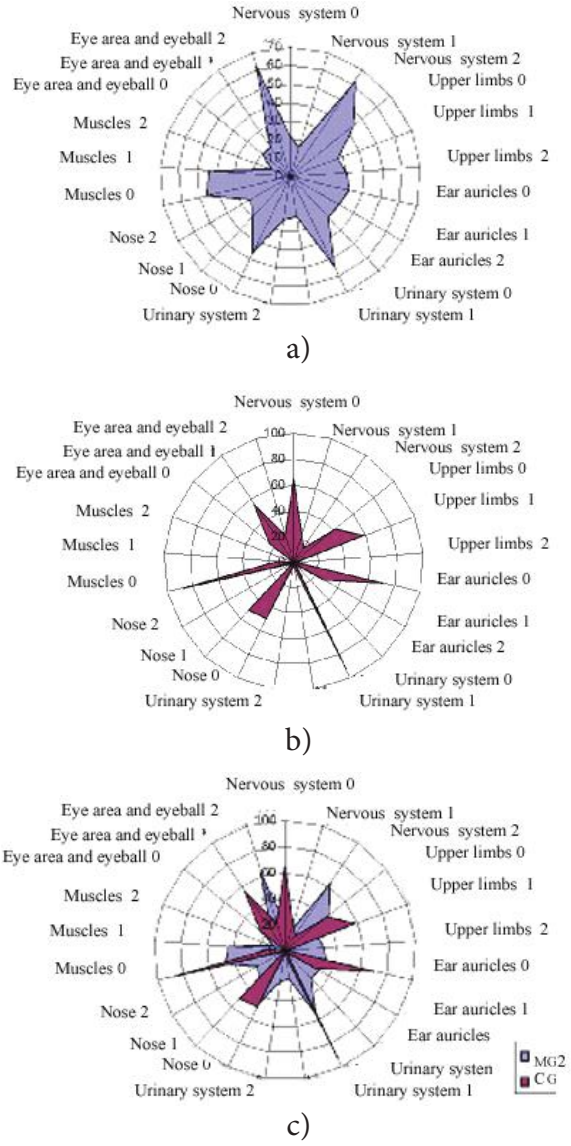


Fig. 9. The diagrams of the distribution of phenotypic traits (nervous system, upper limbs, ear auricles, uriary tract, nose muscle,eye area and the eyeball) of the compared groups MG2 and CG: (a) the diagram MG2; (b) the diagram of CG; (c) the diagrams of MG2 and CG

There is a hypothesis: polymorphic gene variants of mtDNA, which changed their functions during an evolutional process and became negative mutations (from adaptive mutations) and formed predisposition genes, influence on clinical manifestation character and MTChD manifestation. 37 patients with mtDNA polymorphism were examined. Studying association of certain affected systems and organs with certain PmtDNA has allowed to confirm prevalent involvement of energotropny organs in their phenotype: nervous system (78.38% of patients), skeletal (89.16%), digestive system (40.54%), cardiovascular system (35.14%), muscle (43.24%). Significant genetic heterogeneity of lesions is noted and it requires studying polymorphisms kit for differential diagnostics on the assumption of population characteristics of mtDNA haplogroups.

Polymorphism were found in encephalopathies (tRNA – leucine) – 3197 T/C and 3336 T/C; a new mutation (tRNA – lysine) 3624A/G, 3594 C/T, 3705 G/A, 3505 A/G, 3552 T/A; substitution (tRNA – lysine) 8697 G/A, 8806G, 8856 G/A, 8251 G/a, 8701 A/g and a new mutation (tRNA-lysine) 8164 C/T, 8610 T/C, 8614 T/C; AA substitution (tRNA-lysine) syn, thr/ala, pro/leu, ala/thr.

Disorders of muscle system, gastrointestinal tract, skeletal lesions, ophthalmoplegia, cardiopathy, neurosensory deafness have almost such degree of genetic geterogeneity.

These data analysis confirms from the one hand prevalent lesions of nervous system in MTChD, which is associated with PmtDNA and other energotropny organs and systems and from the other hand – the presence of genetic heterogeneity phenomenon (the same clinical profile in different PmtDNA), which negative mutations have. Received data confirm PmtDNA significance in clinical signs formation of PmtDNA, influence presence of neutral nucleotide substitutions on clinical signs character of MTChD.

Received data estimation gives an opportunity to suppose that found clinical signs in patients with MTChD associated with PmtDNA correspond signs range (according to all lesions); which energy metabolism disorder has: these signs are clinically polymorphic, multisystemic, and genetically geterogenic that is due to etiopathogenetic mechanisms and form polyorganic continuum of MTChD (encelopathy, deafness, peripheral neuropathy, myopathy, scoliosis, diabetes, cardiopathy, hepatomegaly, joint dysplasia, respiratory deficiency) (fig.10).

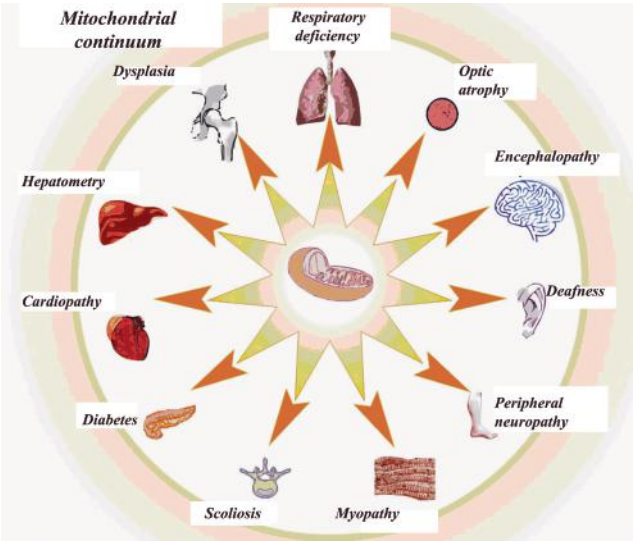


Fig.10. Mitochondrial continuum

It is possible to suppose that mitochondrial balance disorder is formed as a result of pathological mutation influence in mitochondrial or nuclear DNA or in nuclear and mitochondrial, therefore, phenotype character depends on nosological form and involvement degree of organs and systems depends not only on mutation type but also on mtDNA percent (this mtDNA has mutations) (heteroplasmy phenomenon).

These data support the mentioned hypothesis: the information about folate cycle role, its key enzyme – MTHFR and methionine as a universal donor of methyl groups in gene expression regulation was a reason of frequence estimation of polymorphic variants C677 MTHFR/ A66G MTRR. 581(89.1%) from 652 examined patients with different hereditary diseases had polymorphic variants. The main clinical signs of homozygous compounds were presented by cardiovascular pathology, psychomotor development delay, reproductive dysfunction of a family. Geterozygotic compound of these polymorphisms had more range of clinical manifestations that is an evidence of multiorgan affection.

Patients with genotype 677T MTHFR/ 66AG MTRR had significant affections of veins of various localization.

The main clinical signs of 677T MTHFR Hmzgt and 66AG Htzgt carriers were characterized by ectomorphy, dark hair, «sharp" facial features, scoliosis, marble skin, normal intellect, the presence of creative abilities. A registration of such signs as additional to present hereditary disease has suggested syntropy presence «disease conclomerates", meganosology, diseases family, a combination of two and more pathological conditions of diseases, syndromes in one patient. Syntropy is not accidently naturally specific phenomenon, which has evolutionally genetic basis (V.P. Puzurev, 2010, E.Y. Grechanina, R.V. Bogatureva, 2011).

Patient follow-up with different types of hereditary pathology and, first of all, with energy metabolism disorder has shown signs presence of different hereditary disorders in 91 probands.

Profound studying disease history, life anamnesis, genealogy, phenotypic estimation of these patients has allowed us to find several constitutes of clinical manifestations formation of disease. There was the presence of factors in each case and these factors had a character of triggers, mediators and mutation, which were associated with the main disease. Such distribution of exo – and endogenic factors in disease formation has lead to the mechanism search of pathological conditions development and studying gene polymorphism problem as an additional source of gene mutations, in order to define a role methylation disturbance in disease manifestation.

Unique functions of methionine are that it takes participation in transmethylation reaction; is a donor of methyl groups, takes participation in synthesis of biologically active substrates and nucleonic acids; is an methyl acceptor for 5-methylenehydrofolate-homocysteine-methyltransferase (methioninesynthase). Methionine is an irreplaceable amino acid, a component of aminoacyl tRNA biosynthase, metabolism component of glycine, serine, treonine, histidine, methionine, selenoamino acid, tyrosine.

It has been proved that re-methylation disorder (methionine formation from homocysteine), that is a result of MTHFR enzymes deficit, causes pathological condition development such as: aterosclerosis, aterotrombosis closure defects of neural tube, infarcts, nondisjunction of chromosomes in the oogenesis. Studying geno – and phenotypic associations was conducted on the basis of data analysis of selective screening of predisposition genes C677T MTHFR / A66G MTRR in 1938 patients with different forms of hereditary pathology.

Clinical significant differences were defined in carriers of genotype variants MTHFR and MTRR. There was the highest a frequence of lesions of central nervous and cardiovascular system, which were followed by genotypes Htzg / Hmzg and Htzg / Htzg C677T MTHFR and N / Htzg A66G MTRR. The fact of that the influence of these genotypes on pathology formation of leading body systems as pathogenic genes confirm aforesaid data.

Nosological units range has been formed by common disease as well as by monogenic and chromosomal diseases. Clinical signs of deficit of folate cycle enzymes in patients with common diseases were found in 572 (29.5%), and NBO in 492 (25, 4%), and cardiovascular disease in 252 (13.0%), and monogenic disease – in 159 (8.2%), chromosomal syndromes in 102 (5.2%), CTD 95 (4.9%), re-methylation disorder in 86 (4.4%), MTChD – in 76 (3.9%), thromboembolism – in 32 (1.6%), epilepsy – in 24 (1.2%), varicose disease in 21 patients (1.08%), hereditary gastrointestinal diseases in 10 (0.5%),organic aciduria in 9 (0.4%), Duchenne muscular dystrophy – in 6 (0.3%), autism, in 2 (0.1%). Clinical manifestations were compared in patients, who are carriers of both types of polymorphisms (mitochondrial and nuclear DNA) (MG3).

Patients from MG2 and MG3 had skin lesion. Pigmentic spots, angiotelectasia, basal cell nevi were frequent and more expressed against the background of changed methylation. Mesodermal dysplasias in folate cycle deficit were independent in spite of syntropy. Presented data (fig.11, table 12) show that the changes of different organs are not similar and have certain independence, adding their features to MTChD phenotype.

Received data highlight a high frequence of sceletal disorders in the both groups. The majority of patients from both groups has eye changes, that corresponds to clinical signs.

Neck and spine changes occurred in MG3 more

often. More than one third of patient from the both groups had cardiovascular disorders with a slight preponderance in MG2. 54,05% (MG2) and 31,87% (MG3) had muscle lesion, that is possibly related to an adaptive role of combined polymorphisms. Patients of the both groups had urinary system lesion with a slight preponderance in MG3 (tables 8.9).

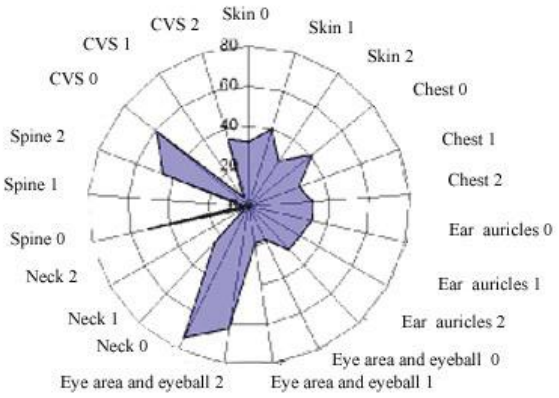
Received data coincide with A.V. Smolyannikova's characteristics – the founder of syntropy meaning: «The second disease has its own pathogenetic features, has a character of independent nosologic unit, which requires special therapeutic actions".

Flayling T.M. et al, 2007, Casas S.P., 2004, V.P. Puzurev, 2010, E.Y. Grechanina, R.V. Bogatureva, 2011, confirmed this opinion.

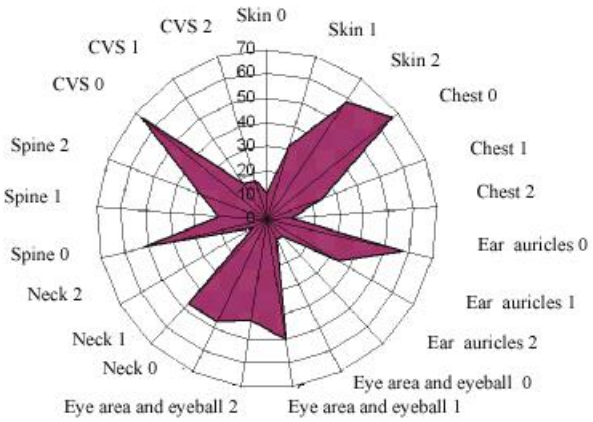
Practical recommendations

Received data have allowed us to make clinical scheme of patient and algorithm of differential diagnostics of MTChD.

Classical and modern research methods in differential diagnostics of MTChD have increased its efficiency up to 93% and have allowed us to develop diagnostic algorithm. And pathogenetic correction development has become important in the absence of etiological treatment in the world (this correction was used in a daily practice).



a)



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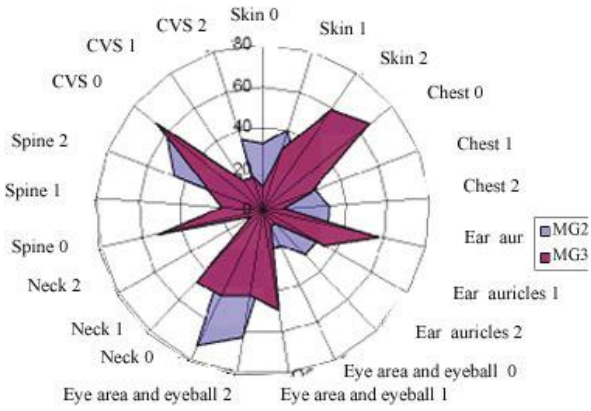
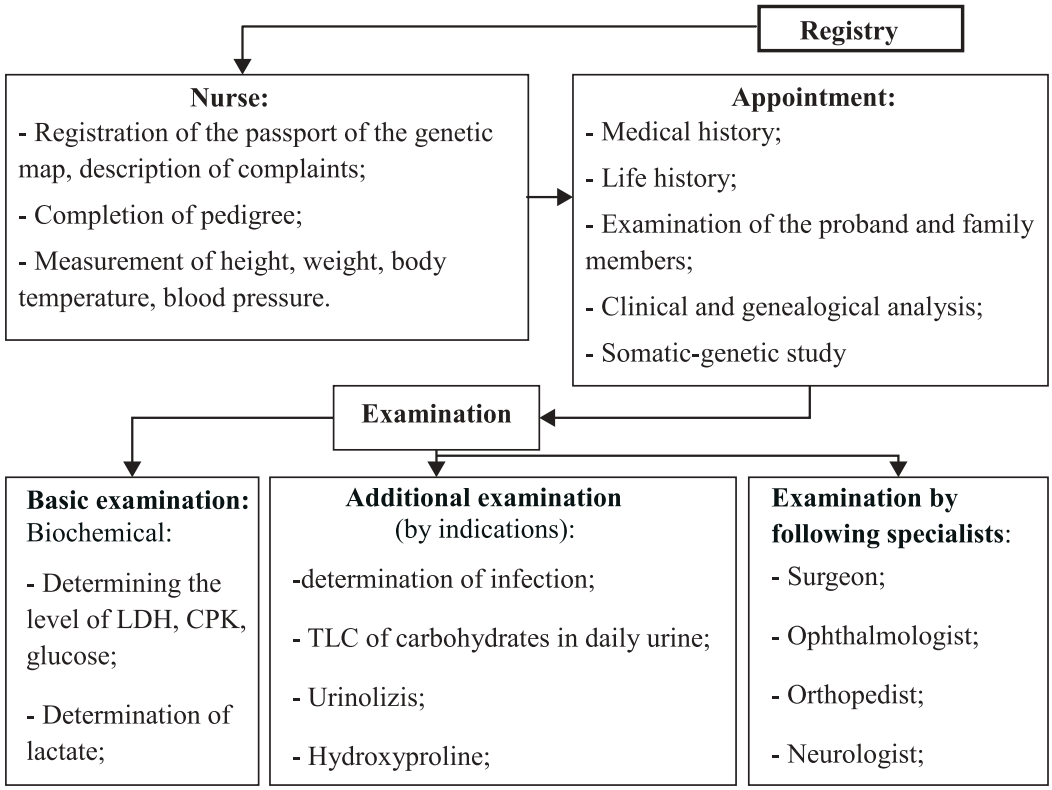


c)

d)

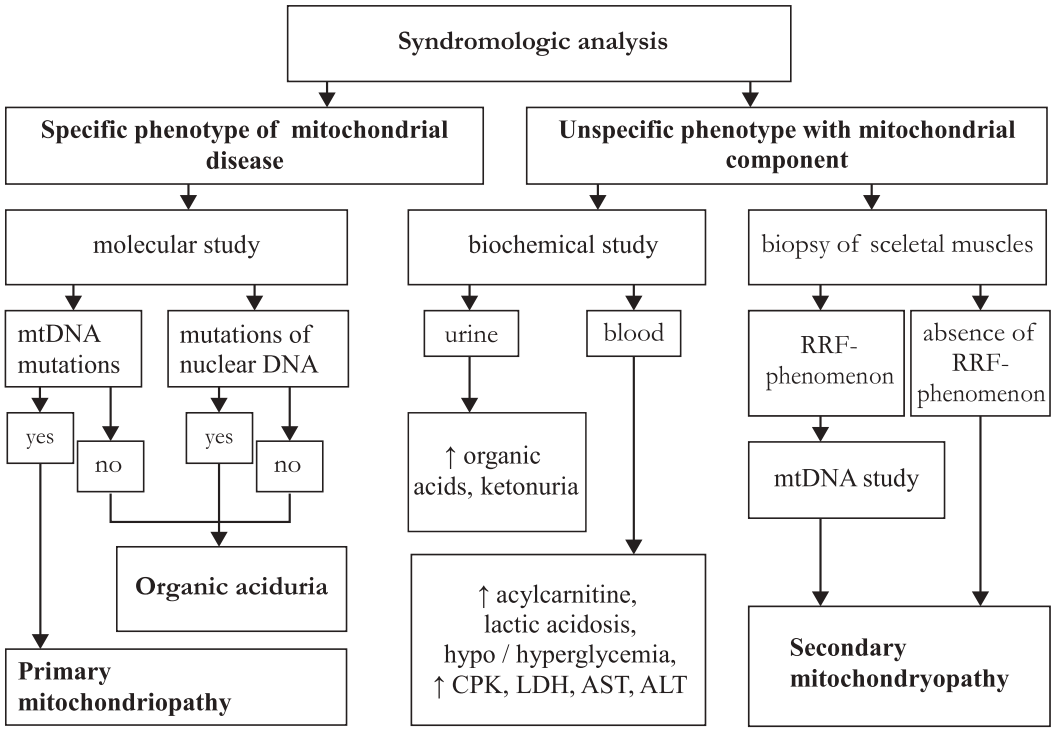
Fig. 11. The diagrams of the distribution of phenotypic features (skin, chest, ears, eye area and eye apple, neck, spine, CVS) of the compared groups MG2 and MG3: a) the diagram of MG2; (b)thr diagram of MG3;(c) the diagrams of MG2 and MG3;(d) thepatient with hamartosis and MTHD

CLINICAL SCHEME OF THE PATIENT
with mitochondrial dysfunction

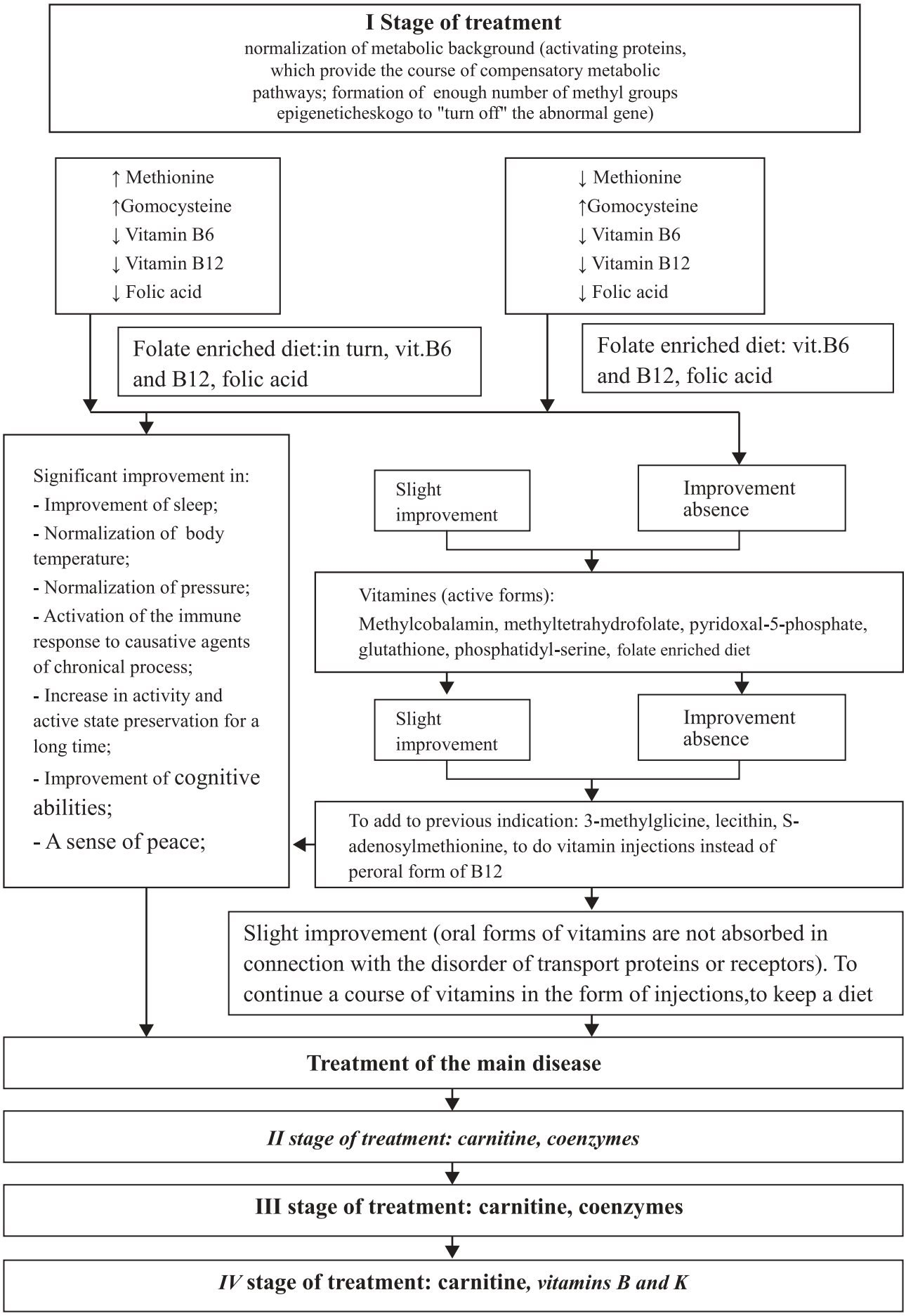


All therapeutic methods and drugs are appointed taking into account individual sensitivity of the patient to the corresponding drugs.

MTChD diagnostics algorithm



Received data evidence that the determination of population characteristics of mtDNA polymorphisms, analysis of individual genomes of mtDNA in probands with a high risk of MTChD and epigenetic status estimation, which (this status) is connected with folate cycle functions, are a valid approach for geno – and phenotypic corrections determination and differential diagnostics of mitochondrial disorders. And the henomenon of genotypic as well as phenotypic syntropy is characteristic of mitochondrial disorders.


I Stage of treatment
normalization of metabolic background (activating proteins, which provide the course of compensatory metabolic pathways; formation of enough number of methyl groups epigeneticheskogo to "turn off" the abnormal gene)
↑ Methionine
↑Gomocysteine
↓ Vitamin B6
↓ Vitamin B12
↓ Folic acid
↓ Methionine
↑Gomocysteine
↓ Vitamin B6
↓ Vitamin B12
↓ Folic acid
Folate enriched diet:in turn, vit.B6 and B12, folic acid
Folate enriched diet: vit.B6 and B12, folic acid
Significant improvement in:
- Improvement of sleep;
- Normalization of body temperature;
- Normalization of pressure;
- Activation of the immune response to causative agents of chronical process;
- Increase in activity and active state preservation for a long time;
- Improvement of cognitive abilities;
- A sense of peace;
Slight improvement
Improvement absence
Vitamines (active forms):
Methylcobalamin, methyltetrahydrofolate, pyridoxal-5-phosphate, glutathione, phosphatidyl-serine, folate enriched diet
Slight improvement
Improvement absence
To add to previous indication: 3-methylglicine, lecithin, S-adenosylmethionine, to do vitamin injections instead of peroral form of B12
Slight improvement (oral forms of vitamins are not absorbed in connection with the disorder of transport proteins or receptors). To continue a course of vitamins in the form of injections,to keep a diet
Treatment of the main disease
II stage of treatment: carnitine, coenzymes
III stage of treatment: carnitine, coenzymes
IV stage of treatment: carnitine, vitamins B and K

Conclusions

Original comprehensive approach has been developed for differential diagnostics of mitochondrial dysfunctions, which includes: the estimation of some population genetic features of populations, the determination of genetic epidemiology of mtDNA polymorphisms and polymorphic gene variants of folate cycle features of «point" mutations carriers and polymorphisms. And this approach gives an opportunity to confirm developed concept in polymorphisms influence on clinical manifestation of MTChD.

Characteristics of the main haplogroups of mtDNA have been composed. The estimation of haplotypes frequences has shown eurospecific haplogroups presence: H (33,5%), V (5,4%), J (11,7%), T (6,7%), U (20,9%), Y (2,1%), W (2,1%), X (2,5%), N (1,2%), total prevalence of HUJT is 72,8%. Eurospecific component was 95,6%, mongoloid – 2%.

Genetic epidemiology of haplotypes in patients with clinically established diagnosis of MTChD was characterized by presence of a smaller specific weight of eurospecific haplogroups (84%) – H pre-V, V, J, T, U, I, X, N, W, their frequences were 24%, 2,0%, 2,0%, 12,0%, 16,0%, 18,0%, 2,0%, 2,0% and 8,0%. Found Asian haplogroups C and A had the frequence of 4%. Higher frequence of haplogroups T, U, X, possibly, is due to an instability of positions 16189 and 16294, which has direct relation to genetic background formation.

Clinical genetic features of mtDNA polymorphisms carriers were characterized by multiorgan course, progreduated influence, clinical polymorphisms, genetic heterogeneity and prevalent involvement of energotropny organs and systems – nervous (62,16% of patients), muscle (42,24%), ophtalmological (62,16%), cardiovascular (35,14%), skeletal (38,0%), digestive (40,54). 75 patients (36,5%) had clinical signs of classical mitochondrial MERRF, MELAS, NARP, Leigh, Kearns-Sayre, Leber syndromes. 92 patients (45,31%) had syntropy phenomenon in which «disease conglomerate" had its specificity.

There is higher multiorgan affection in mtDNA tRNA polymorhisms – lysine: 8697G / A; 8860G; 8701G / A; 8856G / A; 8860 (CRS) 8251G / A; 8472C / T; 8448T / C; 8994G / A; 8337T / C; 8794C / T; 8584G / A; 8701 / G and in amino acid substitution of tRNA – lysine (Syn, thr / ala, pro / leu, met / val, met / thr, his / tyr, ala / thr), encephalopathies were more often associated with tRNA – lysine polymorhism and the new mutations (tRNA – leucine) (3624 A / G; 3594C / T; 3705G / A; 3505 / G; 3552T / A). Muscle, digestive, ophtalmological, cardiovascular, endocrine systems affection was more often associated with tRNA lysine polymorphism. These data confirm clinical significance of mtDNA polymorhisms as negative mutations in formation of clinical signs of MTChD.

Clinical genetic, molecular genetic, mathematical, statistical analysis of examination results of patients with MTChD, associated with mtDNA «point" mutation and certain syndromes has revealed a severe affection of CNS (central nervous system) (82,67% of patients), of urinary system (76,0%),of digestive (84,0%), of nervous (88,0%), of subcutaneous tissue (53,33%), of spine (52,0%), of face (hypomimia) (64,0%), that is an evidence of diagnosis truth and involvement possibility in MTChD pathogenesis not only specific for mutational syndrome, but also for genetic surroundings in MTChD pathogenesis: mutations F124L and E145G ND5, which have changed functionally important sites involved in protons transfer, have led to the change of proton channel and have greatly influenced on phenotype of Leigh syndrome and ,more likely, have become a cause of mutations in mother's fetal cells.

The main clinical signs of polymorphic gene variants C677T MTHFR and A66G MTRR have been defined, the real and virtual phenotypes, which differ depending on polymorphisms character reflecting different actions of mutation, have been made. Phenotype of C677T MTHFR carriers was characterized by dolichostenomelia, skeletal anomalies, high intellect, trombophilia risk in 87,5 % of patients, while polymorphism A66G MTRR in 85,3% of patients had endocrinopathy features – hyperstenichnost prevalence with a short neck, prevalent skin pigmentation, psychic disorders.

Phenotypic features distribution in patients – carriers of mtDNA polymorphisms and polymorphic gene variants of folate cycle has estimated a high Kramer's coefficient in skin affection, its frequence was 88,01%, while among patients –carriers of only mtDNA polymorphisms – 67,57%. The both groups had muscle affection (99,55%), that is an evidence of nosologic independence preservation and simultaneous doubling of clinical manifestation in syntropy.

Developed continuum of clinical signs of MTChD, clinical course of patient with MTChD, diagnostics algorithm of MTChD and a scheme of comprehensive treatment, have allowed to increase diagnostics efficiency up to 93% and to receive an stable remission in a great number of patients with MTChD.

Carried out study has shown the influence of mtDNA polymorphisms on MTChD expression, which is a result of adaptive role substitution on pathogenic against the background of changed methylation as the main genome modificator in result of folate cycle function disorder and certain triggers presence, that shows the way of early diagnostics and an adequate correction of MTChD.

Studying populational characteristics of mtDNA polymorphisms, analysis of individual mtDNA genomes, epigenetic status of folate cycle is a valid approach for differential diagnostics of MTChD.

Grechanina E.Y.

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ВИВЧЕННЯ ВПЛИВУ ПОЛІМОРФІЗМІВ мтДНК І ВАРІАНТІВ ПОЛІМОРФНИХ ГЕНІВ C677T MTHFR ТА A66G MTRR НА КЛІНІЧНІ ПРОЯВИ МІТОХОНДРІАЛЬНОЇ ДИСФУНКЦІЇ

Резюме. Дослідження концепції уточнення діагнозу мітохондропатія засноване на оцінках популяційно-генетичних характеристик – частотних поліморфізмів мтДНК, поліморфних варіантів генів і ферментів фолатного циклу. Знайдено варіабельні положення з високою волатильністю і частотою мутацій, які впливають на виникнення спорадичних мутацій. Показано, що для населення України характерно такий розподіл генотипів та частот алелей генів MTHFR (C677T, A1298S, G1793A); MTRR (A66G); RFC-1 (G80A), який характеризується високою часткою мутантного алеля гомозиготи MTRR (A66G) і 66G, що обумовлено високою частотою уражень центральної нервової системи. Характер клінічних ознак поліморфізму мтДНК-носіїв – поліорганность, прогрaдієнт, клінічний поліморфізм, генетична гетерогенність первинних затримок енерготропів. Встановлено окремі нозологічні форми мітохондропатій – синдроми Лі, Лебера, Кайрнса-Сейра, Синдром MERRF (міоклонічна епілепсія з рваними м'язовими волокнами), синдром MELAS (мітохондріальна енцефаломіопатія, лактатацидоз, інсультоподібні епізоди), NARP (нейропатія, атаксія і пігментний ретиніт), підтверджені клініко – генетично, морфологічними, біохімічними, ферментативними, молекулярно-генетичними методами. Описано основні клінічні особливості носіїв поліморфних варіантів генів C677T MTHFR і A66G MTRR. Ці асоціації мітохондропатії з інвалідністю повторно метіліруют метіонін як можливий прояв

зміненого епігенетичного статусу. Виявлено явище сінтропії і доведено, що вплив на експресію мтДНК поліморфізмів мітохондропатій обумовлено заміною адаптивної ролі по відношенню до зміненого метилювання в якості основного модифікатора генома при порушенні фолатного циклу і наявності певних тригерів.

Ключові слова: мітохондріальна дисфункція; полімофізми мтДНК; поліморфні варіанти генів фолатного циклу; реметилювання метіоніну.

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ИЗУЧЕНИЕ ВЛИЯНИЯ ПОЛИМОРФИЗМОВ мтДНК И ВАРИАНТОВ ПОЛИМОРФНЫХ ГЕНОВ C677T MTHFR И A66G MTRR НА КЛИНИЧЕСКИЕ ПРОЯВЛЕНИЯ МИТОХОНДРИАЛЬНОЙ ДИСФУНКЦИИ

Резюме: Концепция исследования уточняющего диагноза митохондропатия основана на оценках популяционно-генетических характеристик – частотных полиморфизмов мтДНК, полиморфных вариантов генов и ферментов фолатного цикла. Найдены вариабельные положения с высокой волатильностью и частотой мутаций, которые влияют на возникновение спорадических мутаций. Показано, что для населения Украины характерно такое распределение генотипов и частот аллелей генов MTHFR (C677T, A1298S, G1793A); MTRR (A66G); RFC-1 (G80A), который характеризуется высокой долей мутантного аллеля гомозиготы MTRR (A66G) и 66G, что обусловлено высокой частотой поражений центральной нервной системы. Характер клинических признаков полиморфизма мтДНК-носителей – полиорганность, проградиент, клинический полиморфизм, генетическая гетерогенность первичных задержек энерготропов. Установлены отдельные нозологические формы митохондропатий – синдромы Ли, Лебера, Кайрнса-Сейра, Синдром MERRF (миоклоническая эпилепсия с рваными мышечными волокнами), синдром MELAS (митохондриальная энцефаломиопатия, лактатацидоз, инсультоподобные эпизоды), NARP (нейропатия, атаксия и пигментный ретинит), подтвержденные клинико-генетическими, морфологическими, биохимическими, ферментативными, молекулярно-генетическими методами. Описаны основные клинические особенности носителей полиморфных вариантов генов C677T MTHFR и A66G MTRR. Эти ассоциации митохондропатии с инвалидностью повторно метилируют метионин как возможное проявление измененного эпигенетического статуса. Обнаружено явление синтропии и доказано, что влияние на экспрессию мтДНК полиморфизмов митохондропатий обусловлено заменой адаптивной роли по отношению к измененному метилированию в качестве основного модификатора генома при нарушении фолатного цикла и наличии определенных триггеров.

Ключевые слова: митохондриальная дисфункция; полимофизмы мтДНК; полиморфные варианты генов фолатного цикла; реметилирование метионина.

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