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ORIGINAL ARTICLE

Molecular and biochemical study of glutaric aciduria type 1 in 49 Russian families: nine novel mutations in the *GCDH* gene

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Abstract

Glutaric aciduria type 1 (GA1, deficiency of glutaryl CoA dehydrogenase, glutaric acidemia type 1) (ICD-10 code: E72.3; MIM 231670) is an autosomal recessive disease caused by mutations in the gene encoding the enzyme glutaryl CoA dehydrogenase (*GCDH*). Herein, we present the biochemical and molecular genetic characteristics of 51 patients diagnosed with GA1 from 49 unrelated families in Russia. We identified a total of 21 variants, 9 of which were novel: c.127 + 1G > T, c.471_473delCGA, c.161 T > C (p.Leu54Pro), c.531C > A (p.Phe177Leu), c.647C > T (p.Ser216Leu), c.705G > A (p.Gly235Asp), c.898 G > A (p.Gly300Ser), c.1205G > C (p.Arg402Pro), c.1178G > A (p.Gly393Glu). The most commonly detected missense variants were c.1204C > T (p.Arg402Trp) and c.1262C > T (p.Ala421Val), which were identified in 56.38% and 11.7% of mutated alleles. A heterozygous microdeletion of the short arm (p) of chromosome 19 from position 12,994,984–13,003,217 (8233 b.p.) and from position 12,991,506–13,003,217 (11,711 b.p.) were detected in two patients. Genes located in the area of imbalance were *KLF1*, *DNASE2*, and *GCDH*. Patients presented typical GA1 biochemical changes in the biological fluids, except one patient with the homozygous mutation p.Val400Met. No correlation was found between the *GCDH* genotype and glutaric acid (GA) concentration in the cohort of our patients.

Keywords Glutaric aciduria type 1 · *GCDH* gene · Microdeletions · Chromosomal microarray analysis · Neonatal screening

Introduction

Glutaric aciduria type 1 (GA1, glutaryl-CoA dehydrogenase deficiency, glutaric acidemia type 1; ICD-10 code: E72.3; MIM 231670) is an autosomal-recessive disorder caused by mutations in the gene encoding the enzyme glutaryl CoA dehydrogenase (*GCDH*) (Boy et al. 2017). The human *GCDH* gene (OMIM*608801) has been mapped to chromosome 19p13.2, spans ~7 kb, and comprises 12 exons, of which the first is non-coding. A deficiency of the enzyme leads to GA and 3-hydroxyglutaric acid (3-HGA) accumulation in tissues and body fluids, with a predominantly neurotoxic effect

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on subcortical structures. Organic acids can be detected by gas chromatography (GC/MS), and glutaryl-carnitine (C5DC), can be identified by electrospray ionization/tandem mass spectrometry (MS/MS) (Boy et al. 2017). Reportedly, GA1 can be classified into two types based on the level of excreted GA: the high excretion form (GA >1000 mmol/mol creatine) and the low excretion form (GA < 1000 mmol/mol creatine) (Lisyova et al. 2017).

GA1 is a rare inherited metabolic disorder with an estimated worldwide incidence of 1:100,000 live newborns in western Europe (Boy et al. 2017). Five genetic isolates are known to show a high carrier frequency (up to 1:10) and incidence (up to 1:250) (Boy et al. 2017). In Japan, the incidence is estimated to be 1:210,000 live newborns (Mushimoto et al. 2011). The incidence and carrier frequency are unknown in the Russian population.

GA1 usually manifests in early childhood at the ages of 3 to 36 months (peak: 6 to 18 months). The presentation and progression of the disease are variable, ranging from asymptomatic to catastrophic encephalopathy forms. GA1 usually presents before 18 months of age and is usually triggered by infection, with mild or severe acute encephalopathy, striatal degeneration and movement disorder (most often acute dystonia). At a presymptomatic stage, diagnosis is suggested clinically by macrocephaly and specific radiological features: necrotic lesions of basal ganglia develop, which lead to extrapyramidal symptoms (e.g., dystonia, choreoathetosis). Possible secondary complications include feeding difficulties, developmental and speech delays, and chronic aspiration syndrome (Boy et al. 2017).

More than 240 different mutations have been reported in the *GCDH* gene. Although almost all mutations are patient-specific, several common mutations have been identified, including p.Ala421Val in the Amish community, IVS1 + 5G T in Canadian Oji-Cree Indians, and p.Glu365Lys in Irish travelers (Boy et al. 2017). The most frequent mutation in the European population is p.Arg402Trp, and IVS10-2A > C is relatively common in China and Taiwan (Mushimoto et al. 2011).

Materials and methods

Subjects

We studied 51 patients (from 49 families; 29 boys and 22 girls) who had been diagnosed with GA1 based on their clinical presentation, urinary organic acid profiles and/or blood acylcarnitine analysis. The diagnoses were confirmed via *GCDH* gene analysis. The clinical data were available for 47 GA1 patients. Clinical data are summarized in Supplemental 1.

Our work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Informed consent was obtained for experimentation from all patients. This work has been approved by the local ethics committee – «Committee on Biomedical Ethics».

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. All study participants provided informed consent, and the study design was approved by the appropriate ethics review board. We have read and understood journal's policies, and we believe that neither the manuscript nor the study violates any of these. There are no conflicts of interest to declare.

Metabolite analyses

GA1 diagnosis is based on results of urinary organic acid testing and/or acylcarnitine analysis in dried blood spots (DBS).

MS/MS analysis of acylcarnitines in DBS was performed using «NeoGram Amino Acids and Acylcarnitines Tandem Mass Spectrometry Kit» (Perkin Elmer, Finland). Analysis of amino acids and acylcarnitines is carried out on a Sciex 3200 QTrap quadrupole tandem mass spectrometer (ABSciex, USA) with positive ionization in electrospray. The concentrations of amino acids and acylcarnitines were determined as butyl esters by the method of deuterated internal standards. The concentrations of amino acids and acylcarinitines were calculated automatically by comparing the peak intensities of the analyzed compounds with the intensities of internal standards using the NeoGram (Perkin Elmer Life and Analytical Science, Wallac OY, Finland) and ChemoView (ABSciex, USA) programs (Kurkina et al. 2016).

The urinary organic acids were extracted by diethyl ether/ethyl acetate, derivatized and analysed by GC/MS 7890A/5975C (Agilent Technologies, USA) with HP-5MS (Kurkina et al. 2016).

DNA sequencing

Genomic DNA was extracted from blood samples with the use of Diatom DNA Prep reagent kits (Biocom, Russia), following the manufacturer's recommendations. Sequencing of the *GCDH* gene was performed using an ABI Prism 3500 (Applied Biosystems), following the manufacturer's protocol. PCR primers were designed based on the reference sequence of the *GCDH* gene NM_000159.3 including all encoding exons and flanking intronic sequences.

Chromosomal microarray analysis (CMA)

The copy number analysis of the *GCDH* gene, including a copy number of their exons, was made using a Cytoscan XON Assay (ThermoFisher, USA). DNA extraction and

processing were performed according to the manufacturer's instructions. The data were analyzed with reference to the recommendations of the American College of Medical Genetics and Genomics (ACMG), and performed using ChAS 4.0 software and an in-house database.

Assessing the pathogenicity of GCDH missense mutations

The nomenclature of molecular variants followed the Human Genome Variation Society guidelines (HGVS, www.hgvs.org/mutnomen), using the human *GCDH* (*608801; glutaryl-CoA dehydrogenase) cDNA sequence: NM_000159.3 from the Human Gene Mutation Database (HGMD, www.hgmd.cf.ac.uk). The NetGene2 Server (http://www.cbs.dtu.dk/services/NetGene2) was used to determine splicing alterations. The pathogenicity of previously undescribed missense-mutations was predicted using the SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), and MutationTaster (http://www.mutationtaster.org) tools. The evolutionary conservation of interspecies was assessed using UniProt server (http://www.uniprot.org/). To determine the effects of nucleotide sequence changes on the protein structure, 3D-modeling of the identified amino acid substitutions was performed using CMBI (http://www.cmbi.ru.nl/hope/) software (Venselaar et al. 2010).

Results

Clinical characteristics

The GA1 diagnosis was confirmed in the 51 patients (from 49 families; 29 boys and 22 girls). The clinical data were available for 47 Russian GA1 patients. The disease manifestation and clinical course were primarily characterized by delays in development and speech, macrocephaly, dystonia, ataxia, pyramidal tract abnormalities, and seizures. Cranial MRI/CT demonstrated basal ganglia damage, hypoplasia of the temporal lobes, white matter pathology, and arachnoid cysts. Two patients were asymptomatic: one of which was detected in a newborn screening pilot study, and the other in a family screening study (patient 43 and 46, respectively). Cases 36 and 37, cases 42 and 43 were siblings (Suppl. 1).

Disease manifested before the age of 24 months in 36 cases, in 4 cases - at 3–5 years of life. The triggers were infectious diseases, vaccinations, brain injuries. Acute development of the disease was noted in two-thirds of patients, subacute – in 8 cases. Five of these patients subsequently, in a few weeks or months, developed an acute encephalopathic crisis with aggravated clinical symptoms. Macrocephaly was observed in 35 patients. The main clinical manifestation in patients was generalized dystonia. Epileptic seizures were observed in 24 patients, more often as a complication of acute encephalopathic crisis. Regression of motor development and a delay in cognitive development were noted in most patients and were an indicator of the severe course of the disease. Despite the treatment, these patients still have a pronounced neurological deficit. In all patients (for whom neuroimaging data were available) subarachnoid spaces in the frontotemporal-parietal regions were enlarged. Bilateral temporal arachnoid cysts were observed in 24 patients, signs of basal ganglia lesion – in 29 patients and leukoencephalopathy – in 39 patients (Suppl. 1).

Biochemical diagnosis

The elevated levels of GA, 3-HGA, and C5DC in body fluids are the major biochemical markers of GA1 (Boy et al. 2017) (Suppl. 1).

In 46 GA1 patients, MS/MS showed elevated levels of GA1-specific biochemical markers (C5DC). C5DC values were increased up to 0.54–8.28 umol/l (normal range: 0.0–0.45 umol/l).

GA in urine was elevated in 47 patients, with levels ranging from 101.00 to 21,710.30 mmol/mol creatinine (normal range: 0–2 mmol/mol creatinine). In one case 48, the GA concentration was normal (1.85 mmol/mol creatinine).

The 3-HGA measurement in urine is limited by overlapping chromatographic peaks of 3-HGA and α-ketoglutaric acid, which significantly complicate the quantitative analysis. Extracted ion chromatograms for three characteristic ions of 3-HGA (m/z = 259; m/z = 185; m/z = 349) were used to extract a pure signal of 3-HGA from co-eluting components. The 3-HGA analysis was elevated in 37 patients, with concentrations ranging from 5 to 6867 mmol/mol creatinine (normal range 0–2 mmol/mol creatinine) (Chen et al. 2009; Bergmann et al. 2018; La Marca and Rizzo 2011).

The data available for 47 patients indicated that 2 were classified as low-excretors, defined by a urinary GA below 100 mmol/mol creatinine, while the remaining 45 patients were considered high-excretors with GA levels above this value (Lisyova et al. 2017).

Molecular genetic diagnosis

We conducted a molecular genetic study of samples from the 51 patients with biochemical and clinical data characteristics of GA1. We found 21 homozygous or compound heterozygous mutations in the *GCDH* gene (NM_000159.3.3) in 42 patients, of which 9 had previously not been reported (Suppl. 1, Table 1). In eight patients, only one heterozygous mutation was detected. In five of these patients, the study was not completed because an insufficient amount of biomaterial was provided. CMAs were performed for three patients with one heterozygous mutation. Heterozygous microdeletions of the short

Table 1 Mutations identified in 51 patients diagnosed with GA1 in Russia

Exon/ Intron	Mutation	The distribution of mutations of the total number of alleles in the gene *GCDH*	Allele frequency in GnomAD browser	RUS	ACMG score	Conclusion
2	c.79delG*	1 (1.0%)	Not found	*0*	–	–
IVS3	**c.127 + 1G > T** *	1 (1.0%)	Not found	*0*	*PVS1,* PM2, PM3, PP3, PP4	Pathogenic
4	c.219delC	7 (7.3%)	Not found	–	–	–
	c.161 T > C (p.(Leu54Pro)) *	1 (1.0%)	Not found	*0*	PM2, PM3, PP3, PP4	Probably pathogenic
6	c.382C > T (p.Arg128Term)	1 (1.0%)	Not found	–	–	–
	c.471_473delCGA *	1 (1.0%)	Not found	*0*	*PVS1,* PM2, PM3, PM4, PP3, PP4	Pathogenic
7	c.583G > A (p.Ala195Thr)	3 (3.1%)	Not found	–	–	–
	c.531C > A (p.(Phe177Leu)) *	3 (3.1%)	Not found	*0*	PM2, PM3, PP3, PP4	Probably pathogenic
	c.647C > T (p.(Ser216Leu)) *	1 (1.0%)	Not found	*0*	PM2, PM3, PP3, PP4	Probably pathogenic
8	c.743C > T (p.Pro248Leu)	1 (1.0%)	1.27e-4	–	–	–
	c.705G > A (p.(Gly235Asp)) *	1 (1.0%)	Not found	*0*	PM2, PM3, PP3, PP4	Probably pathogenic
9	**c.898 G > A (p.(Gly300Ser))** *	1 (1.0%)	Not found	*0*	PM2, PM3, PP3, PP4	Probably pathogenic
10	1045G > A (p.Ala349Thr)	1 (1.0%)	3.19e-5	–	–	–
11	c.1204C > T (p.Arg402Trp)	53 (55.2%)	3.15e-5	–	–	–
	c.1205G > C (p.(Arg402Pro)) *	1 (1.0%)	Not found	*0*	PS1, PM2, PM3, PP3, PP4	Pathogenic
	c.1178G > A (p.(Gly393Glu)) *	1 (1.0%)	Not found	*0*	PM2, PM3, PP3, PP4	Probably pathogenic
	c.1198G > A (p.Val400Met)	2 (2.0%)	1.04e-4	–	–	–
	c.1169G > C (p.Gly390Ala)	1 (1.0%)	Not found	–	–	–
	c.1157G > A (p. Arg386Gln)	1 (1.0%)	Not found	–	–	–
	c.1168G > C (p.Gly390Arg)	1 (1.0%)	1.41e-4	–	–	–
12	c.1262C > T (p.Ala421Val)	10 (10.4%)	1.49e-4	–	–	–

Mutation distribution in exons and the adjoining intron regions in the *GCDH* gene, and the analysis of the pathogenicity of new mutations with the use of bioinformatics programs

*- mutations detected in this study

GnomAD: The Genome Aggregation Database (https://gnomad.broadinstitute.org/)

MAF: Minor allele frequency

RUS: The Russian 870 exomes database (an in-house database)

ACMG score: Scores recommended by the American College of Medical Genetics and Genomics

arm of chromosomes 19–12,994,984–13,003,217 (8233 b.p.) and 12,991,506–13,003,217 (11,711 b.p.) were detected in patients 26 and 6, respectively. The genes located in the area of imbalance were *KLF1*, *DNASE2*, and *GCDH*. No changes were found by CMA in patient 17, possibly due to the nucleotide changes being located deep in the introns or in the regulatory region.

In silico analysis of novel mutations

Multiple sequence alignment of *GCDH* and its homologue protein sequences revealed that the sites where the mutations were observed were highly conserved, implying that the observed mutations are not tolerated by protein structure and function. This result was corroborated by the predictions made by the different tools, which unanimously predicted the mutations as pathogenic (Table 1).

The novel deletion c.471_473delCGA is not a frame shift mutation, and results in the loss of one codon and change of an amino acid sequence. These may lead an amino acid sequence changing from the 287 to 294 amino acid region, which is responsible for substrate binding, and thus may lead to lack of the functional domain necessary for substrate binding.

A novel substitution which probably affects the splicing site in intron 3 (c.127 + 1G > T) was found. Tang et al. demonstrated that the mutation (c.127 + 1G > A) of this consensus donor site results in abnormal splicing of intron 3, whereby the screening of control samples did not reveal any carrier of c.127 + 1G > A (Tang et al. 2000). Thus, it is highly probable that c.127 + 1G > T would lead to the abnormal splicing of intron 3.

The novel pathogenic substitution c.161 T > C (p.(Leu54Pro)), c.531C > A (p.(Phe177Leu)), c.647C > T (p.(Ser216Leu)), c.705G > A (p.(Gly235Asp)), c.898 G > A (p.(Gly300Ser)), c.1205G > C (p.(Arg402Pro)), c.1178G > A (p.(Gly393Glu)) occur in highly conserved regions of the protein. The bioinformatics analysis based on protein 3D-modeling showed that above mentioned substitutions, may lead to protein folding alterations, disruption of domain interactions, loss of existing hydrogen bonds, formation of new steric bonds, due to different molecule charges, sizes, and polarity, resulting in damage of the protein conformational structure (Fig. 1) (Venselaar et al. 2010).

c.161 T > C (p.(Leu54Pro))

The substitution of Leu54 by Pro is located at the N-terminal domain of the region that is essential for the formation of bonds with other amino acids. The smaller proline size leads to disruption of conformational interactions with the region that regulates protein activity. Thus, the novel pathogenic substitution can cause conformational changes in the protein structure and lead to abnormal signaling between binding and activity domains.

c.647C > T (p.(Ser216Leu))

The novel pathogenic substitution occurs in the middle of the N-terminal domain, close to the central domain. Normal wild-type amino acids form hydrogen bonds with methionine (263 position). The mutant Leu is bigger in size and differs from the wild-type Ser in its hydrophobic properties, which can lead to the loss of existing hydrogen bonds in the protein core and abnormal folding.

c.705G > A (p.(Gly235Asp))

The novel pathogenic substitution occurs in the middle of the N-terminal domain. The mutant Asp is bigger in size, negatively charged (unlike neutral Gly), and more hydrophobic. Gly235 is located relatively close to the protein core. Due to the differences in size and physical properties, the mutant Asp235 can cause altered protein conformation and structural destabilization.

c.898 G > A (p.(Gly300Ser))

The substitution of Gly300 in the C-terminal domain to Ser occurs in the active site. Size differences of the wild type and mutant Ser can result in structural destabilization of this domain region, thus influencing the catalytic activity.

c.1205G > C (p.(Arg402Pro))

Arg402 forms hydrogen bonds with Val133, Asp134, and His399. The difference in molecule size and hydrophobicity can lead to the loss of existing hydrogen bonds. Arg402 also forms salt bridges with Asp134 and Asp396. Charge differences will result in the loss of ionic interactions with Arg402, which, together with the absent hydrogen bonds, can cause altered domain interactions and reduced protein functional activity.

Discussion

Clinical characteristics

The clinical course of GA1 in Russian cohort of GA1 patients (with all types of detected mutations) in the present study was similar to the abundant data from European populations, where newborn screening for this disease has not yet been introduced (Suppl. 1).

Cases 45 and 46 were identified in a pilot neonatal screening project. Diet therapy was carried out from 1 month to 6 years of age, acute encephalopathic crises and neurological symptoms were not observed in the patient (case 46). In case 45 diet therapy was carried out from 1 week (Suppl. 1).

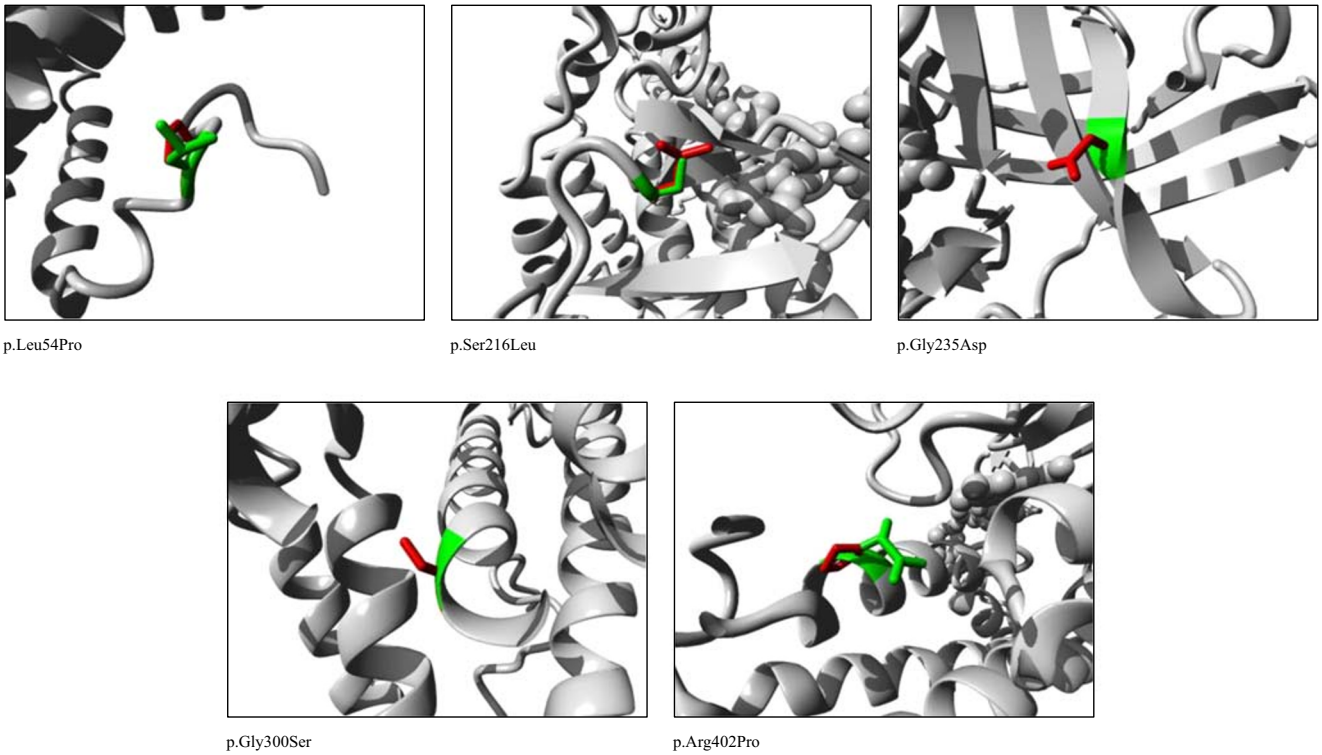

Fig. 1 Analysis of the novel mutations in the *GCDH* gene based on the CMBI web service (http://www.cmbi.ru.nl; red: mutant AA, green: normal AA) (Venselaar et al. 2010)

In two observed patients with a known mutation (p.Arg402Trp) and heterozygous microdeletion on the short arm of chromosome 19, the disease began at the first year of life. In a case 6, acute encephalopathy developed at the age of 9 months; in a case 26, subacute development of hyperkinesis was observed from 8.5 months and subsequent crisis with regression of motor skills at the age of 13 months. Both patients had severe neurological deficits. Three patients with new mutations (cases 16, 17, 27) had the same severe course of the disease.

Five patients with new mutations (cases 3, 22, 29, 31, 35) had a milder phenotype. Epileptic seizures in them are not registered. Cognitive impairment in these patients was either not observed or was not expressed, and the restoration of motor skills allowed, despite the presence of hyperkinesis, to attend educational institutions.

Biochemical diagnosis

Biochemically, GA1 is characterized by the accumulation of GA, 3-HGA, glutaconic acid (not obligatorily) and C5DC in the body fluids (urine, blood, cerebrospinal fluid) and tissues. GA1 patients with normal C5DC levels can be diagnosed by the presence of increased levels of urinary 3-HGA which is considered being the most reliable diagnostic marker. In some patients, the urinary excretion of GA can be completely normal and the glutaconic acid may appear during an acute attack only. Also, it is necessary to consider the possibility of incorrect diagnosis that could be caused by glutaric aciduria type II (multiple acyl-CoA dehydrogenase deficiency) (Lisyova et al. 2017).

Biochemical phenotype (GA, 3-HGA and/or C5DC) in all reported patients was typical to GA1 (Boy et al. 2017). Our cohort was characterized by abnormal excretion of GA, 3-HGA, C5DC with different magnitude of excretion.

Based on biochemical findings, the amount of excreted GA and enzyme activities, two subgroups of GAI patients can be distinguished. Low-excretors are with the residual enzyme activity up to 30% and are defined by a lower level of urinary GA excretion (below 100 mmol/mol creatinine) and high excretors with a complete loss of enzyme activity typically excrete high amounts of GA usually above 100 mmol/mol creatinine (Lisyova et al. 2017).

The data available for 49 patients in our cohort indicated that 2 were classified as low-excretors, defined by a urinary GA below 100 mmol/mol creatinine, while the remaining 48 patients were considered high-excretors with GA levels above this value (101.00 and 1.85 mmol/mol creatinine, cases 48 and 41, respectively).

Molecular genetic diagnosis

More than 240 different *GCDH* gene mutations have been reported thus far. (http://www.hgmd.cf.ac.uk). The review of published literature and various mutation databases indicated that no significant rearrangements have been described to date in the *GCDH* gene.

In the present study, 21 mutations were found in 49 families in Russia. Nine were novel substitutions, including c.127 + 1G > T, c.471_473delCGA, c.161 T > C (p.(Leu54Pro)), c.531C > A (p.(Phe177Leu)), c.647C > T (p.(Ser216Leu)), c.705G > A (p.(Gly235Asp)), c.898 G > A (p.(Gly300Ser)), c.1205G > C (p.(Arg402Pro)), c.1178G > A (p.(Gly393Glu)). The pathogenicity of the novel substitutions was assessed with reference to the recommendations of ACMG (Table 1).

One of the most common *GCDH* gene mutations is c.1204C > T (p.Arg402Trp), with a frequency of 12–40% in western Europe (Boy et al. 2017). The frequency of this mutation has been shown to be 18.8% (of studied alleles) in India (Kruthika-Vinod et al. 2017), 57.0% - in patients in Spain (Boy et al. 2017), and 50.0% - in Poland (Boy et al. 2017), while this mutation is absent in Japan (Mushimoto et al. 2011). Published data suggests that this mutation is more prevalent in European populations. The mutation c.1204C > T (p.Arg402Trp) being the most frequently detected in Russian patients (56.38% mutant alleles), is consistent with European data (Table 1).

It is noteworthy that the second most frequent mutation in our study was c.1262C > T (p.Ala421Val) (11.70%) (Table 1), which was found to be highly prevalent in the Amish community in Pennsylvania (Boy et al. 2017). A possible explanation for this finding is that a migration occurred in Russia in 1803–1808 yy from the Alsace province (German to 1697 and 1871–1919 yy) to the southern part of Russia where they formed a Russian-German community. Some Amish, who originated from Alsace and Lorraine, migrated to the New World in the nineteenth century.

The frequency of these two mutations in the databases of different populations was very low, i.e., <0.0005 for p.Arg402Trp and < 0.0001 for p.Ala421Val mutations. In the present study of 870 Russian exomes, these two most frequent mutations were not detected (Table 1).

The limitations of Sanger sequencing can hinder the identification of gross gene rearrangements (insertions, deletions, duplications) and regulatory mutations deep in the introns. The search of mutations in these gene regions requires the use of additional methods, such as chromosomal microarray, multiplex ligation-dependent probe amplification, etc. In the present study, we have found a heterozygous microdeletion of the short arm (p) of chromosome 19 from position 12,994,984–13,003,217 (8233 b.p.) and from position 12,991,506–13,003,217 (11,711 b.p.) in two patients. The genes located in the area of imbalance were *KLF1*, *DNASE2*, and *GCDH*. An analysis of published findings revealed that this is the first case of microdeletions detected by CMA in GA1 patients, and that these mutations were in compound heterozygous state with the most common mutation being p.Arg402Trp.

The association between the *GCDH* genotype and GA concentrations has previously been reported in the literature (Lisyova et al. 2017; Schillaci et al. 2016). Clinical phenotype does not seem to be related to the mode of onset of the disease and also does not seem to be directly related to the severity of the biochemical phenotype (Radha Rama Devi et al. 2017). Interestingly, in two patients with the p.Ala195Thr/p.Arg402Trp genotype, GA concentrations were lower compared with other cases (182.00 and 250.00 mmol/mol creatinine, respectively) and patient with p.Gly390Ala/p.Arg402Trp genotype was identified as low-excretor (101.00 mmol/mol creatinine). The mutations c.1213A > G (p.Met405Val), c.1198G > A (p.Val400Met), c.680G > C (p.Arg227Pro), and c.856C > T (p.Pro286Ser), which are associated with low-excretors of GA1, with both p.Met405Val and p.Val400Met being much more common in patients of African origin compared to the general global population (Schillaci et al. 2016). Besides, false-negative results have been reported in newborns with the mutation p.Met405Val (Marti-Masso et al. 2011). In some cases, GA1 patients with homozygous p.Val400Met mutations presented normal urine GA and 3-HGA levels (Marti-Masso et al. 2011). In our cohort, a normal GA level and 3-HGA level slightly higher than the reference were observed in patient case 48, who presented the homozygous mutation p.Val400Met. These biochemical data may be due to *GCDH*-p.Val400Met variant presents in vitro a high residual activity (Ribeiro et al. 2019).

Conclusion

This is the first report on the molecular profile of Russian patients diagnosed with GA1. The clinical course of GA1 in the presently studied Russian cohort was similar to that of many other European populations, where newborn screening has not yet been introduced for this disease. In Russian patients, the p.Arg402Trp mutation in *GCDH* gene was highly prevalent, with nearly 30% of patients being homozygous for this variant. The prevalence of the mutation p.Ala421Val may be explained by the historical background of the populations. The contribution of microdeletion may be underestimated in the spectrum of mutations in GA1 and can influence the severity of the phenotype.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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