MEDICAL AND BIOLOGICAL ASPECTS RELATED TO ASSESSMENT OF IMPACTS EXERTED BY RISK FACTORS

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UGT1A1 GENE MUTATION AS A MARKER INDICATING THERE IS A HIGH RISK OF GILBERT'S SYNDROME: THEORETICAl AND APPLIED ASPECTS

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Gilbert's syndrome is a widely spread multi-factor pathology which is to a great extent genetically determined. Its basic etiological factor is lower activity of a liver enzyme, UDP-glucuronosyltransferase A1, caused by mutations in UGT1A1 gene. Functional disorders in the liver cause dyspepsia and concurrent acute and chronic diseases in the digestive system. The research goal was to substantiate the necessity and possibility to conduct mass examinations of population with molecular and genetic analysis of UGT1A1 gene in order to reveal Gilbert's syndrome. The authors performed molecular and genetic examination of UGT1A1 gene rs8175347 marker in 132 people living in Kemerovo region (population sampling) as well as in 71 patients who were supposed to have Gilbert's syndrome (clinical sampling).

*Frequency of *28/*28 mutant genotype of UGT1A1 gene associated with Gilbert's syndrome amounted to 13.6 % in the population sampling and it is quite consistent with previously published data. Therefore, a considerable rate of population includes people with potential or already revealed Gilbert's syndrome. Age structure of patients in the clinical group with *28/*28 genotype revealed there was a wide spread of an age at which the diseases was first detected due to its apparent manifestation; age varied from 4 to 71 years with its modal value being equal to 15 years. Basing on the obtained data, it is suggested to implement mass examinations aimed at revealing Gilbert's syndrome at its prenosological stage; such examinations can be based on molecular-genetic technologies. When children aged 7-10 are comprehensively examined, they can also undergo genetic diagnostics aimed at revealing any mutations in UGT1A1 gene. Obtained genetic data can be taken into account by medical personnel with relevant medical specializations when they determine strategies aimed at preventing and curing Gilbert's syndrome.*

Key words: *Gilbert's syndrome, UDP-glucuronosyltransferase A1, UGT1A1, rs8175347, mutations in a gene, genotype, molecular and genetic examination, genetic diagnostics.*

Gilbert's syndrome (GS) is the most frequent type of genetically determined pigmentary hepatosis. Icteritiousness of skin, sclera, and mucous tunics is its typical external evidence. The disease can also lead to various dyspeptic occurrences and asthenovegetative syndrome. Symptoms of the pathology are usually caused by physical overstrain or infectious diseases, and they can appear after starvation or low-calorie diet as well as after taking certain medications [1, 2]. Increased bilirubin concentration in blood is a basic laboratory parameter; it occurs primarily due to an indirect fraction. Both physical and biochemical indicators of the disease are variable, therefore they can't be considered reliable and sufficient to put a diagnosis, especially when it comes to children who haven't reached adolescence [3].

Basic GS signs are transitory and aren't considered to directly lead to grave damage to the liver. But still GS is often accompanied with other gastrointestinal tract diseases [4], and cholelithiasis can be a probable remote outcome of the syndrome in some patients [5, 6]. This trend becomes even more

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apparent in case of any concomitant diseases. Thus, when GS is combined with sickle-cell anemia, it makes cholelithiasis much more probable [7, 8]. Gilbert's syndrome in infants with hemolytic states causes much greater risks of complications and death in the neonatal period [2].

Biochemical and genetic basics of GS are now well-established. Lower activity of UDP-glucuronosyltransferase A1 in case of the disease is determined either by changes in relevant *UGT1A1* gene expression or by structural modifications in the enzyme itself. In the first case there is usually a change in number of dinucleotide repeats *TA* in the gene promoter section (*rs8175347* polymorph marker). Thus, if a "wild type" allele **1* is characterized with six tandem repeats *TA*, then in case of GS-related mutations their number increases up to 7 (allele **28*) or 8 (allele **37*). Another variation in the promoter section is characterized with fewer repeats *TA*, usually 5 (allele **36*) and leads to increased *UGT1A1* activity without any pathological signs [9, 10]. It was detected that genotype **1/*28* carriers and especially **28/*28* carriers tended to have on average higher bilirubin concentrations in their blood serum than homozygotes **1/*1* [11–13].

There are very scarce data on *UGT1A1* genetic polymorphism as well as on its physiological and biochemical signs in population in Russia [14, 15]. Besides, Gilbert's syndrome frequency is unknown and there are no data on how many people or what specific population groups run risks of this pathology. It makes additional population-genetic research on *UGT1A1* truly vital.

This research can be considered relevant and practically significant as there is a necessity to work out efficient medical and diagnostic algorithms aimed at detecting multi-factor pathologies, such as Gilbert's syndrome, on the basis of the latest achievements in genetics and allied sciences.

Our research goal was to substantiate the necessity and possibility to perform mass health examinations among country population aimed at detecting Gilbert's syndrome via molecular and genetic analysis of *UGT1A1* gene.

Data and methods. We performed our examinations on two groups of people living in Kemerovo region; all the examined people were Caucasians (predominantly Russians). The population group was made up of personnel and patients who underwent a prophylactic medical examination at Kemerovo regional clinical hospital, both sexes (68 females and 64 males), the data are given in the Table. We didn't take the examined people's health into account. This sampling was applied to perform population-genetic analysis of *rs8175347* polymorphic marker in *UGT1A1* gene.

Table

Characteristics of the examined groups

Group	n	Age, years			
		M ± S.E.	Me	Mo	min-max
Population	132	36.4±0.61	36	38	23–51
Clinical	71	34.3±2.30	30	15	4–71

Note: n is a group volume; M is a simple mean; S.E. is a standard error of the mean; Me is median; Mo is mode; min-max are variation limits.

The clinical sampling included patients who were treated at Kemerovo regional clinical hospital (38 females and 33 males) and had been previously sent to a medical-genetic consultation to have a medical-genetic examination in relation to supposed Gilbert's syndrome. Polymorph section *rs8175347* in *UGT1A1* gene was analyzed in all patients and a conclusion on homozygosity as per a mutant allele **28* was made; it confirmed a preliminary diagnosis was correct. This group allowed to examine certain age aspects related to GS manifestations and detection.

Venous blood samples were taken from all the examined people in an in-patient department and EDTA was applied as an anticoagulant. DNA was extracted out of whole blood on "K-Sorb" columns produced by "Syntol" company according to the manufacturer's instructions. PCR-amplification of polymorph section *rs8175347* in *UGT1A1* gene was performed with a commercial reagent kit manufactured by "Litech" LLC. We detected allele**1* (a wild type, 6 *TA*-repeats in a promoter section of

UGT1A gene) and **28* (mutation, 7 *TA*-repeats). To detect PCR results, we applied horizontal electrophoresis of amplification products in 3% agarose gel with ethidium bromide used as a colorant.

We statistically analyzed initial data and calculated basic selective parameters for quantitative variables. Frequencies of marker rs8175347 alleles and genotypes in *UGT1A1* gene were calculated as fractions of their overall number in a sampling. Qualitative variables distributions were compared with χ^2 criterion in "STATISTICA 6.0". A discrepancy was considered to be authentic at $p<0.05$.

Results and discussion. When discussing genetic factors that cause GS risks, we should first of all assess prevalence of mutations in *UGT1A1* gene which are associated with the pathology. **1/*1* (47.0%) prevailed in the examined population group that included Caucasians living in Kemerovo region (Table 2). Frequency of minor allele **28* associated with a pathologic state amounted to 33.3%. A fraction that belonged to the rarest genotype **28/*28* associated with Gilbert's syndrome was equal to 13.6%. The established ratio of the genotypes didn't have any authentic discrepancies from the expected one in accordance with Hardy-Weinberg equilibrium ($\chi^2 = 0.674$; $p = 0.714$). It proves there is no apparent deadaptation in any genotype and a selection against this genotype in the examined population.

Table 2

Population and genetic peculiarities of marker *rs8175347* in *UGT1A1* gene in different Caucasian ethnic groups

Ethnic group	*UGT1A1* genotypes *(rs8175347)*, %			Allele frequency **28*, %	Source
	**1/*1*	**1/*28*	**28/*28*		
Russians	47.0	39.4	13.6	33.3	Own data
Russians	40.4	50.0	9.6	34.6	[14]
Russians	42.4	51.7	5.9	31.8	[15]
Croats	39.9	49.8	10.2	35.1	[16]
Italians	43.9	39.8	16.3	36.2	[17]
Dutch	44.2	43.7	11.9	33.7	[18]
Spaniards	40.0	51.0	9.0	34.5	[19]
Caucasians in the USA	46.6	43.1	10.0	31.6	[20]

Pathologic genotype frequency which we detected is rather high and it requires further confirmation based on other independent research. Data on population and genetic peculiarities of *UGT1A1* gene in population in Russia are scarce. Recently some research results have been published on *UGT1A1* polymorphism in a sampling which included people living in the south of Russia (Rostov-on-Don) [14]. According to the obtained results, **28/*28* genotype fraction amounted to 9.6%, and allele **28* frequency was even higher than we detected in our research. We should also note that this examined sampling, strictly speaking, can't be considered a population one as it was made up of oncologic patients who suffered from colorectal cancer. In other research, Shatalova E.G. et al. [15] detected frequency of *rs8175347* alleles and genotypes in healthy Russian females. Frequency of **28/*28* homozygotes amounted to 5.9% only, and a fraction of **28* allele was equal to 31.8%.

We analyzed some other works that focused on examining *UGT1A1* polymorphism; according to them, a fraction of a clinically significant allele **28* usually exceeds 30% but doesn't reach 37% among Caucasians [16–20]. It corresponds to a homozygote genotype **28/*28* frequency being, as a rule, about 10% or a bit higher. Such frequency of a minor allele is close to its critical value when fractions of homozygotes **1/*1* and heterozygotes are practically the same. Therefore there was a common regularity detected in different examined groups, that is, comparable frequencies of two genotypes, **1/*1* и **1/*28*, usually with insignificant prevalence of **1/*1* variant.

We can conclude that our data in general are consistent with results obtained in previous research. A fraction of potential or already detected patients with Gilbert's syndrome among Caucasians who are carriers of *UGT1A1 *28/*28* genotype usually exceeds 10%.

High frequency of homozygote *UGT1A1* mutation carriers detected in various research requires developing and implementing specific algorithms into medical practices that will allow to perform mass health examinations in order to detect population groups that run ele-

vated GS risks; such algorithms should be based on high-precision pathology markers. As it was stated previously, Gilbert's syndrome symptoms usually become apparent when adolescence begins and vary significantly in different patients depending on specific combinations of external influences. In such a situation conventional diagnostic techniques turn out to lack precision and make for a longer period during which the disease is diagnosed.

But at the same time, all the contemporary knowledge on Gilbert's syndrome etiology and a role played by mutations in *UGT1A1* gene allow to propose a reliable algorithm for GS diagnostics based on molecular and genetic approaches. Timely detection of mutant **28/*28* genotype carriers, ideally as early as at the prenosological stage already, enables adjusting a life style which a patient pursues in order to prevent the pathology from its manifestation or making possible harm to a patient's health as minimal as its only possible.

To determine an optimal age at which a diagnostic examination should be accomplished, we studied the sampling that comprised patients with GS with the diagnoses being given on the basis of clinical manifestations and detection of **28/*28 UGT1A1* genotype (Figure).

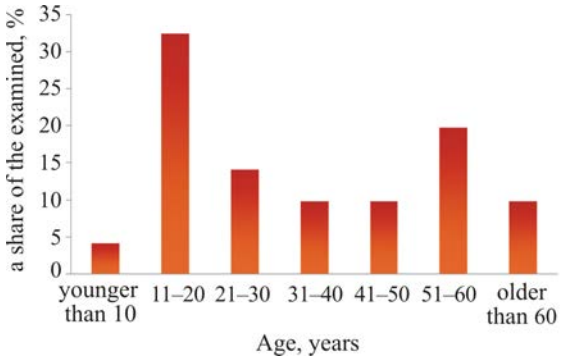

Figure. Age distribution of the examined patients suffering from GS

An age at which GS was diagnosed for the first time can vary significantly, starting from 4 and up to 71 (Table). But at the same time, more than 30% of the examined patients were people younger than 20, and a modal age as per the clinical sampling amounted to 15. It is consistent with previously published observations, according to which basic GS manifestations usually appear when pubescence starts [3, 21]. A fraction of patients in older age groups was slightly lower. The second peak related to GS diagnosis was detected among people older than 50 which can be caused by a decrease in body functional reserves at an older age and combined effects produced by pathogenic factors which appeared in previous years.

Obviously, there are people with GS being diagnosed for the first time in all age groups. The disease manifestations are known to be associated with an individual combination of pathogenic factors, peculiarities of a body, lifestyle, diet, etc., and they are un predictable in terms of the ontogenetic aspect [1–3]. Nevertheless, we can assume that an inherited decrease in *UGT1A1* function in most cases will sooner or later become apparent due to impacts exerted by either endogenous or exogenous factors. Besides, we can also assume there are individuals in a population with various manifestations of the pathology caused by **28/*28 UGT1A1* genotype carrying who haven't ever applied to a medical organization.

As it was mentioned above, when GS is diagnosed too late and there is no relevant therapy, it can cause elevated health risks for a patient [5–8, 21]. In our opinion, an age before pubescence starts is an optimal one at which GS should be detected as any symptoms of the pathology are usually absent thus early. Such check-ups can be performed during regular mass health examinations among school children.

According to the Order issued by the RF Public Healthcare Ministry "On a procedure for medical examinations of minors including those performed before entering an educational establishment and during studies"[1] minors are to be examined on a regular basis at a certain age. Schoolchildren usually undergo

[1] On a procedure for medical examinations of minors including those performed before entering an educational establishment and during studies: The Order by the RF Public Healthcare Ministry issued on December 21, 2012 No. 134bn [Web-source] // Garant: information and legal database. – URL: https://www.garant.ru/products/ipo/prime/doc/70255102/ (date of visit February 14, 2019).

a comprehensive medical examination at 7, 10, as well as 14, 15, 16, and 17 years. Notably, the existing system of medical examinations for minors doesn't include a consultation and examination by a gastroenterologist; given that, GS can be detected only when its first symptoms become apparent and a patient starts to complain about health.

A child usually starts school at 7 and additional risk factors that can cause GS appear at this moment. They are physical and psychoemotional strain and organized meals at a school. Before a child starts school, his or her parents usually can control the timetable, a child's diet and how regularly he or she eats, but when a child goes to school, the task becomes rather complicated. Children undergo preventive medical examinations at 7 and 10 and visit some medical specialists during them. They also have their blood analyzed; it could be an optimal moment to diagnose GS via examining *UGT1A1* gene mutations. Small aliquots of whole blood could be a DNA source.

A methodical part in a molecular-genetic examination can be implemented on the basis of the previously described approaches with a domestic instrumental base and test-systems so that requirements related to replacing imports of medical materials and equipment are met. A diagnostic procedure is going to be cheap and easy to perform thus being available for mass application [22].

Mass genetic examinations aimed at detecting Gilbert's syndrome will allow to divide all people into 2 groups. **1/*1* и **1/*28* genotypes carriers will be assigned into "conditional standard"; **28/*28* genotype carriers, into "a risk group as per Gilbert's syndrome". A "standard" can be only "conditional" as we can't completely exclude other, rarer mutations among people from this group; these mutations can appear in *UGT1A1* gene sections which haven't been examined so far and they can also lead to GS symptoms occurrence. But still, there are too few such people as it was detected in previous research on various ethnic groups [11, 20].

People from "risk group as pert Gilbert's syndrome" should be observed thoroughly by a gastroenterologist and their genetic data should be included into their overall case history. As genetic diagnostics is supposed to be performed among children, most of them can have no apparent GS signs. And public healthcare specialists should first of all communicate health risks to potential patients and perform efficient activities aimed at preventing any GS manifestations. It primarily concerns making children have responsible attitudes towards their lifestyle and diet. It is necessary to exclude factors that stimulate the pathology development; such factors include nervous and physical strain, acute chills, abundant low-calorie, spicy, or fried food in a diet as well as starvation or irregular meals etc.

Specific medical activities chosen for each individual patient will depend on peculiarities related to the pathology manifestations. Apart from relevant therapeutic measures, a physician should thoroughly analyze reasons that caused the disease manifestations in order to eliminate them completely. Obviously, knowledge on genetic predisposition to Gilbert's syndrome, preventive activities and timely relevant therapy can lead to a substantial improvement in life quality for most people from the risk group.

Conclusion. We conducted population-genetic research on *rs8175347* marker in *UGT1A1* gene among people living in Kemerovo region; the research revealed high frequency of **28/*28* mutant genotype which was associated with Gilbert's syndrome (13.6%). Obviously, a significant share of the population are potential patients or people with already diagnosed GS. It requires immediate measures to be taken to provide early detection of people with GS among those who run genetic risks and to implement prophylaxis and therapeutic activities in order to prevent the disease manifestations in them.

We proposed an algorithm for mass health examinations aimed at detecting GS at its prenosological stage; such examinations can be based on molecular-genetic technologies. When children aged 7 or 10 undergo their obligatory comprehensive medical examination as it is fixed in the existing regulatory documents, they can additionally have *UGT1A1* mutations diagnosed in them. To do

that, medical experts can use laboratory equipment and test-systems produced in Russia, and it will allow to accomplish mass health examinations among children in a shortest period of time.

All obtained results can be taken into account by relevant medical specialists so that they can determine further necessary actions aimed at preventing and curing Gilbert's syndrome. Correct determination or necessary adjustment of a lifestyle, behavior, and diet will allow to significantly improve life quality for those people who have genetic predisposition to GS.

The suggested algorithm is a feasible translation medicine model. The same procedure can be applied in relation to other multifactor diseases with well-known genetic component and significant genetic predisposition. Ideally, such activities should underlie a future system of personified medicine which focuses on individual genetic and physiological peculiarities of a particular patient.

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