РЕЗЮМЕ

МАЗЬ ГИАЛУРОНИДАЗЫ ДЛЯ ЛЕЧЕНИЯ ГИПЕРТРОФИЧЕСКИХ РУБЦОВ

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Изучена эффективность гиалуронидазной мази, созданной на основе препарата микробной гиалуронидазы "Билидаза", в лечении постоперационных гипертрофических рубцов. В исследование включены 14 пациентов с постоперационными гипертрофическими рубцами в разных частях лица. Лечение проводили путем введения гиалуронидазной мази ионофорезом в течение 10 дней, каждодневными процедурами. Повторный курс лечения проводился с четырехнедельным интервалом. Оценку состояния рубца осуществляли по "шкале оценки Ванкувера", которая учитывает васкуляризацию, пигментацию и подвижность рубца, боль и зуд.

Результаты исследования показали, что при лечении гиалуронидазной мазью значительно улучшаются функциональные и косметологические показатели рубцовой ткани. До лечения общий средний балл составлял 8,1±0,35, после первого курса лечения - 5,14±0,9, а после второго курса - 0,85±0,9 ($p<0,001$). Использование гиалуронидазной мази может быть рекомендовано также при операциях пластической хирургии для предупреждения формирования постоперационных рубцов.

რეზიუმე

ჰიალურონიდაზის მალამო ჰიპერტროფიული ნაწიბურების მკურნალობისათვის

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შესწავლილია მიკრობული ჰიალურონიდაზას სამკურნალო პრეპარატის "ბილიდაზას" საფუძველზე შექმნილი ჰიალურონიდაზას მალამოს ეფექტურობა პოსტოპერაციული ჰიპერტროფიული ნაწიბურების მკურნალობაში. ჰიალურონიდაზის შემცველი პრეპარატები ფართოდ გამოიყენება სამედიცინო პრაქტიკაში. კვლევაში ჩართული იყო სახის სხვადასხვა ნაწილში ოპერაციის შემდგომი ჰიპერტოფიული ნაწიბურის მქონე 14 პაციენტი. ჰიალურონიდაზის მალამოს შეყვანა ხდებოდა იონოფორეზის საშუალებით. პაციენტებს მკურნალობა უტარდებოდა ყოველდღიური პროცედურებით 10 დღის განმავლობაში. მკურნალობის განმეორებითი კურსი ტარდებოდა 4- კვირიანი შუალედით. ნაწიბურის შეფასება ხდებოდა პიგმენტაციის, ვასკულარიზაციის, ძვრადობის, სიმაღლის, ასევე ტკივილისა და ქავილის მიხედვით „ვანკუვერის შეფასების სკალის" შესაბამისად. გამოკვლევის შედეგებმა აჩვენა, რომ ჰიპერტროფიული ნაწიბურების ჰიალურონიდაზის მალამოთი მკურნალობა მნიშვნელოვნად აუმჯობესებს ნაწიბუროვანი ქსოვილის როგორც ფუნქციურ, ასევე კოსმეტიკურ მახასიათებლებს. მკურნალობამდე შეფასების საშუალო ჯამური ქულები შეადგენდა 8,1±0,35-ს, მკურნალობის ერთი კურსის შემდეგ – 5,14±0,9-ს, ხოლო მკურნალობის ორი კურსის შემდეგ – 0,85±0,9-ს ($p<0.001$). ჰიალურონიდაზის მალამოს გამოყენება ასევე შეიძლება პლასტიკური ოპერაციისშემდგომი ნაწიბურების წარმოქმნის საწინააღმდეგოდაც.

PREVALENCE OF *PAH* MUTATIONS IN GEORGIAN PKU PATIENTS COMPARED TO MOST FREQUENT PAH MUTATIONS IN EUROPEAN POPULATIONS

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Metabolic disorders (MD) are often life-threatening health conditions, which are caused by deficiency or absence of various metabolic enzymes. Majority of MD are from the group of inherited metabolic diseases (IEM). Metabolic disorders differ in types of inheritance pattern. The incidence of MD can vary in different populations. Because of diversity of MD, it is necessary to provide personalized treatment plans for patients. Phenylketonuria (PKU) is one of the most frequent metabolic disorders in the world. In Georgian population its frequency is 1:6060 newborns (Table 1). For European countries the frequency is 1:10000 [1,8,10].

The biggest step in diagnostics of PKU was the discovery of *PAH* gene and *PAH* mutations. Population studies described different mutations and their frequencies for various populations. Studies have revealed more than 900 different mutations in *PAH* gene [5].

Table 1. Total number of annual births and PKU newborns in Georgia

Year	Total newborns	PKU newborns
2004	40388	8
2005	43574	8
2006	44965	11
2007	47464	6
2008	53519	13
2009	59053	6
2010	60021	6
2011	55271	9
2012	56464	11
2013	56845	9
2014	58777	8
2015	60408	10
2016	56149	6
2017	52799	10
2018	42652	8
2019	47903	9

Table 2. Mutation locations on PAH gene

Mutation Type	Location on *PAH*
R408W;Y414C;A403V	Ex12
R252W;P281Q;R243Q;E280K;R261Q;P281L	Ex7
L48S;R53H	Ex2
IVS12+1G>A	int12
IVS10-11G>A	int10
S349P	Ex10
V388M;E390G	Ex11
S67P	Ex3
R178Q	Ex6
L165T;R158Q	Ex5

PKU causes delay of mental developmental stages and other neurological disorders in untreated patients. Symptoms also include pigment deficiency of the skin and eye, seizures and microcephaly. In most countries PKU is diagnosed through newborn screening programs, which test the patients' blood for various metabolic and genetic disorders. The list of tested disorders can vary for different countries. Metabolic screening allows to start the treatment before the symptoms appear. Early diagnosis is a key element for successful treatment of PKU [2-4].

Clinical manifestation of PKU depends on mutation type. Mutations can differ from each other by clinical manifestation, enzyme activity and amount. For diagnostics of *PAH* mutation, mostly molecular methods are used [6,7].

PKU is inherited through autosomal recessive pattern. Mutations on both alleles are necessary for symptoms to develop. Correlation between the genotype and the phenotype is also an important consideration. Different mutations can have different impact on the disease development and its severity. Mutations in same gene can cause classic, moderate and mild forms of disease. The genotype/phenotype correlation is very complex for MD. Gene expression and gene interaction is also an important factor. Different family members with same mutations can develop different levels of symptom severity and different types of clinical manifestation. Symptom development can also depend on the environmental factors [11-13].

Phenylalanine (Phe) is an essential amino acid. A non-essential amino acid tyrosine is produced from Phe metabolism. This metabolic pathway is necessary for production of epinephrine, norepinephrine and melanin. Therefore, Phe is an important amino acid for mental development. Phe was first extracted in 1879 and was synthesized artificially for the first time in 1882. Phe is one of 20 amino acids which are encoded by the standard genetic code for construction of proteins [14-17].

During infancy PKU patients do not differ from the healthy infants, but symptoms appear with time. Affected infants have light skin and eye color. Hyperactivity is one of the possible symptoms in PKU. 2 out of 3 patients develop mental developmental delay. 1 out of 4 patients develop seizure episodes which are hard to treat. Possible symptoms include tremor, microcephaly and liver deficiency [18,19].

In Georgia newborn screening is mandatory. Phe is measured in dry blood spot which is collected after first 24-72 hours of life. By the national guideline normal Phe value in healthy individual is <120 μmol/L. Early diagnosis is crucial for optimal outcome. It is important to prevent the incidence of the disorder in same family. Prenatal diagnostic of the disease is available through noninvasive methods. Molecular genetic methods are considered the golden standard for PKU diagnostics [20-22].

PKU is divided in groups depending on the severity of symptoms and the *PAH* enzyme activity, which is determined by mutation type. Mutations are divided in 3 groups: mild, moderate and classic. Mutations are described in databases by their clinical manifestation [23,24].

The main goal in treatment is to keep Phe levels in normal range (20-120 μmol/L). This is achieved by a restricted diet, which must be maintained during life. Diet therapy starts immediately after the diagnosis. Treated patients remain healthy mentally as well as physically. Elevation of blood Phe level damage brain and can cause impairment in thinking and decision making, as well as social and emotional problems. If diet is not maintained permanently, the symptoms become severe and chronic [25-27].

PKU frequency differs by the country and region. In Europe the incidence of PKU is around 1:10000 newborns, but in some European countries the numbers are higher. In Turkey the frequency is 1:6500 newborns. Finland has a lower frequency of 1:100000. In Georgia frequency of PKU is 1:6060 newborns. Screening for PKU in Georgia started in 2004 [28-30].

In this study we reviewed literature to determine the most common *PAH* mutations in Europe. The aim of the study is to determine the frequencies of these mutations in Georgian population and make comparisons with the European population. Study results are used to confirm or deny the similarities between Georgian and European *PAH* gene mutations and therefore suggest whether European panels are suitable for genotyping Georgian PKU patients.

Material and methods. First step of the study was to determine the most frequent European *PAH* mutations through literature data. Countries include: Germany, Netherlands, Belgium, Switzerland, France, Austria, Poland, Estonia, Liethueva, Czech Republic, Slovakia, Russia, Ukraine, Romenia, Hungary, Denmark, Finland, Sweden, Norway, Island, Great Britain, North Ireland, Croatia, Serbia, Greece, Italy, Spain, Portugal, Turkey, Azerbaijan and Armenia [31-40]. Using data of the mentioned countries most common 20 *PAH* mutations were determined (Table 3).

Biological material was collected from 40 Georgian patients. The study subjects were PKU patients who are registered in Georgian Governmental PKU program. Patients or their legal representatives signed the informed consent about the use of the biological material for the study Diagnoses of PKU were confirmed by molecular methods.

2 mL venous blood was collected in EDTA tubes. Samples were marked and registered. Blood was frozen at -4°C. DNA was extracted from the blood using PureLink Genomic DNA Kit (Thermofisher Scientific K181002) according to the manufacturer's manual. DNA concentration was measured and marked on the tubes. Extracted material was frozen at -20°C.

Table 3. Most common mutations per country

Country	Mutation
Germany	R408W; IVS12+1G>A; Y414C; IVS10-11G>A
Netherlands, Belgium	IVS12+1G>A; R261Q; R158Q
Switzerland, France, Austria	R408W; IVS12+1G>A; R261Q, R158Q; IVS2+5G>C
Poland	R408W; IVS10-11G>A; IVS12+1G>A
Estonia	IVS12+1G>A; R261Q; R252W; R158Q; S349P
Lithuania	R408W; R178Q; A403V
Check Republic, Slovakia	R408W; IVS12+1G>A; R158Q; R261Q; R252W
Russia	R408W; R261Q; P281Q; R252W; R158Q; R261X; R243Q; E280K; IVS10-11G>A
Ukraine	R408W; R158Q; R252W; P281L; Y414C
Bulgaria	R408W; R158Q; IVS10-11G>A; 1089delG
Denmark	R408W; Y414C; IVS12+1G>A
Sweden	R408W; IVS12+1G>A
Norway	R261Q; R408W; Y414C; IVS12+1G>A
Great Britain	R408Q; IVS12+1G>A
Croatia	R408W; P281L; R261Q; E390G
Serbia	L48S; R408W; P281L; E390G; R261Q
Greece	P281L; IVS10-11G>A
Italy	IVS10-11G>A; R261Q; L48S; R158Q
Spain	IVS10-11G>A; A403V; V388M; I165T
Portugal	IVS10-11G>A; R261Q; V388M
Turkey	1066-11G>A R261Q, R252W
Azerbaijan	IVS10-11G>A; S67P; R261Q; R252W
Armenia	IVS10-11G>A; P281L

PCR was conducted using following concentrations: AmpliTaqGold360 (12.5µl), GC Enhancer (2 µl), Primer F (2 µl), Primer R (2 µl), DNA extract (1 µl), Nuclease free water (5.5µl). After amplification, the amplified material was purified with PureLinkPCR Amplification Kit (Thermo Fisher Scientific).

Sanger sequencing was used for detection of *PAH* gene mutations using predesigned primer pairs from Thermofisher Scientific was used (Table 4). Following concentrations were used: BD Terminator3.1 (1 µl), BDT 5X Buffer (0.5 µl), PrimerF (1 µl), amplified material (3 µl), NFW (4.5 µl). Sequencing reaction material was purified again with BigDye XTerminator purification Kit (Thermo Fisher Scientific). Material was loaded in 3500 Genetic Analyzer (Thermo Fisher Scientific) for detection.

Finally, the mutations were identified in the resulting data and their frequencies were analyzed.

Results and discussion. *PAH* mutations were detected on all 80 alleles, clinical diagnose of PKU was confirmed in all 40 patients. Detected mutations in Georgian population was: P281L in 37.5%, IVS10-11G>A in 17.5%, R261X in 10%, L48S in 8.75%, E280K in 5%, R270K in 3.75%, E390G in 3.75% and mutations R252W, IVS12+1G>A, R243Q, R261Q, 1089delG, Y387H, EX5del, IVS7-5T>C, IVS12+1G>A, G171R, IVS2+5G>C each in 1.25% (Table 5). Homozygous forms of PKU were detected in 10 patients. P281L/P281L genotype was detected in 7 patients, IVS10-11G>A/IVS10-11G>A genotype was detected in 3 patients.

The most common mutation from European panel (Table 5) was P281L (37.50%), this mutation is predominant in countries like Croatia, Ukraine, Serbia, Greece, Russia and Armenia. In Armenian and Greek population, it is considered to be the most common *PAH* mutation. Next most frequent mutation is IVS10-11G>A (17.50%) which is most predominant mutation in Greece, Italy, Spain, Portugal, Turkey, Armenia and Azerbaijan. Mutation L48S (8.75%) is common in Serbian and Italian population. E280K (5%) is prevalent in Russia. E390G (3.75%) is common in Russia, Croatia and Serbia. The most frequent mutation for European population is R408W which was not detected in the investigated 80 alleles.

Table 4. Primer pairs (Thermo Fisher Scientific) used in the study

Exon	Pre designed primer pair
12	Hs00126409_CE
11	Hs00126410_CE
7	Hs00735250_CE
6	Hs00126415_CE
5	Hs00746108_CE
3	Hs00817238_CE
2	Hs00552008_CE

Table 5. Frequencies of mutations per allele of Georgian PKU patients

Mutation	N	Frequency
P281L*	30	37.50%
IVS10-11G>A*	14	17.50%
R261X	8	10%
L48S*	7	8.75%
E280K*	4	5%
R270K	3	3.75%
E390G*	3	3.75%
R252W*	1	1.25%
IVS12+1G>A*	1	1.25%
R243Q*	1	1.25%
R261Q*	1	1.25%
1089delG	1	1.25%
Y387H	1	1.25%
EX5del	1	1.25%
IVS7-5T>C	1	1.25%
IVS12+1G>A	1	1.25%
G171R	1	1.25%
IVS2+5G>C	1	1.25%

** - mutations coinciding with the European panel*

Thus, from 20 common mutations of European populations 9 were detected in Georgian patients. Accordingly, 11 common European mutation in 80 alleles investigated by us were not detected. At the same time, detection of the PAH Exons 2, 3, 5, 6, 7, 11, 12 also revealed 9 mutations other than from European panel (Table 5).

Despite this fact, that from 138 today registered PKU patients in Georgia, only 40 (29%) were included in this study, even now it could be suggested that only 9 mutations from the European most frequent mutations panel are suitable for PKU genotyping among Georgian population. At the same time more than half (55 %) of the mutations found in Georgians were not identified as the most common mutations in Europe. This may indicate the necessity in the nearest future for the development of diagnostic panels specific to the Georgian population, including both 9 frequent European PAH mutations and 9 mutations more common for the Georgian population, which will significantly improve the quality of PKU diagnostics in Georgia.

Here, it must be said that the results have been obtained are of an intermediate nature, which propose to continue and complete this research by studying the entire Georgian PKU population.

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SUMMARY

PREVALENCE OF *PAH* MUTATIONS IN GEORGIAN PKU PATIENTS COMPARED TO MOST FREQUENT PAH MUTATIONS IN EUROPEAN POPULATIONS

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The aim of the study was to compare Georgian PAH mutation spectrum to the most frequent European mutations.

Population study publications were reviewed and 20 most frequent European PAH mutations were determined. Mutations were detected in 40 Georgian PKU patients using Sanger sequencing.

PAH mutations were detected on all 80 alleles, clinical diagnose of PKU was confirmed in all 40 patients. Detected mutations in Georgian population was: P281L in 37.5%, IVS10-11G>A in 17.5%, R261X in 10%, L48S in 8.75%, E280K in 5%, R270K in 3.75%, E390G in 3.75% and mutations R252W, IVS12+1G>A, R243Q, R261Q, 1089delG, Y387H, EX5del, IVS7-5T>C, IVS12+1G>A, G171R, IVS2+5G>C each in 1.25%.

Study revealed that the most common Georgian PAH mutations spectrum differs from the European one. 9 out of 18 detected mutations coincide with the European panel. At the same time more than half (55%) of the mutations found in Georgians were not identified as the most common mutations in Europe.

These findings may indicate the necessity for the development of diagnostic panels specific to the Georgian population, including both 9 frequent European PAH mutations and 9 mutations more common for the Georgian population, which will significantly improve the quality of PKU diagnostics in Georgia. The results have been obtained are of an intermediate nature, which propose to continue and complete this research by studying the entire Georgian PKU population.

Keywords: PAH gene, PKU, mutation spectrum, Georgian PKU patients, frequent mutations.

РЕЗЮМЕ

РАСПРОСТРАНЁННОСТЬ НАИБОЛЕЕ ЧАСТЫХ ДЛЯ ЖИТЕЛЕЙ ЕВРОПЫ РАН МУТАЦИЙ СРЕДИ ГРУЗИНСКИХ ПАЦИЕНТОВ С ФЕНИЛКЕТОНУРИЕЙ

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Целью исследования явилось сравнение спектра мутаций в гене РАН у грузинских пациентов с фенилкетонурией с наиболее частыми европейскими мутациями.

Проанализированы публикации популяционных исследований, определены 20 наиболее частых европейских мутаций в гене РАН. В грузинской популяции исследованы 40 грузинских пациентов с фенилкетонурией (ФКУ) путем секвенирования по Сэнгеру.

Клинический диагноз ФКУ подтвержден у всех 40 пациентов. РАН мутации выявлены в 80 исследуемых аллелях. Обнаруженные мутации в грузинской популяции распределены следующим образом: P281L - у 37,5%, IVS10-11G> A - в 17,5%, R261X - в 10%, L48S - в 8,75%, E280K в - 5%, R270K и E390G - в 3,75% случаев. Мутации R252W, IVS12. + 1G> A, R243Q, R261Q, 1089delG, Y387H, EX5del, IVS7-5T> C, IVS12 + 1G> A, G171R, IVS2 + 5G> C выявлены в 1,25% случаев каждая.

Исследование показало, что спектр наиболее распространенных РАН мутаций в Грузии несколько отличается от европейского: 9 из 18 обнаруженных в Грузии мутаций совпадают с европейской панелью. В то же время более поло-

вины (55%) мутаций, выявленных нами, не входят в список самых распространенных европейских мутаций.

Полученные результаты указывают на необходимость разработки диагностических панелей, специфичных для населения Грузии, включающих как 9 частых европейских мутаций PAH, так и 9 мутаций, более характерных для населения Грузии, что значительно улучшит качество диагностики ФКУ в стране. Полученные результаты носят промежуточный характер, что предполагает продолжение и завершение данного исследования путем изучения всей грузинской ФКУ популяции.

რეზიუმე

ევროპაში გამოვლენილი ყველაზე ხშირი PAH მუტაციების სიხშირის შეფასება ქართულ პოპულაციაში ფენილკეტონურიით

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კვლევის მიზანს წარმოადგენს ქართული PAH მუტაციების სპექტრის შედარება ყველაზე ხშირ ევროპულ მუტაციებთან.

ჩატარდა პოპულაციური კვლევების მიმოხილვა და გამოვლინდა 20 ყველაზე ხშირი ევროპული PAH მუტაცია. ქართული პოპულაციის PAH მუტაციები იყო იდენტიფიცირებული სენგერის სეკვენირების მეთოდით.

გამოკვლეულ 40 პაციენტში ფენილკეტონურიით (PKU) გამოვლენილი მუტაციები განაწილდა შემდეგნაირად: P281L - 37.5%, IVS10-11G>A - 17.5%, R261X - 10%, L48S - 8.75%, E280K - 5%, R270K - 3.75%, E390G - 3.75%. R252W, IVS12+1G>A, R243Q, R261Q, 1089delG, Y387H, EX5del, IVS7-5T>C, IVS12+1G>A, G171R, IVS2+5G>C მუტაციებისგან თითოეული დაფიქსირდა შემთხვევების 1.25%-ში.

კვლევამ აჩვენა, რომ ქართული პოპულაციისათვის დამახასიათებელი PAH მუტაციების სპექტრი განსხვავდება ევროპულისგან. 18-დან მხოლოდ 9 გამოვლენილი მუტაცია ემთხვევა ევროპულ პანელს. ამავდროულად, ნახევარზე მეტი (55%) მუტაციებისა, რომელიც გამოვლინდა ქართულ პოპულაციაში, არ შედის ვროპული პოპულაციისათვის დამახასიათებელ 20 ყველაზე ხშირ მუტაციების სიაში.

აღნიშნული შედეგები მიანიშნებს სპეციფიკური სადიაგნოსტიკო პანელის შემუშავების აუცილებლობაზე, რომლიც გამოყენებული იქნება ქართული პოპულაციისათვის, აღნიშნულ პანელში შევა როგორც 9 ყველაზე ხშირი ევროპული მუტაცია, ასევე 9 მუტაცია, რომელიც გამოვლინდა ქართულ პოპულაციაში, რაც მნიშვნელოვნად გააუმჯობესებს ფენილკეტონურიის დიაგნოსტიკას საქართველოში. მიღებული შედეგები არის შუალედური ხასიათის, შესაბამისად, კვლევის გაგრძელება მთლიანი PKU პოპულაციაში მნიშვნელოვანია.

НЕЙРО-ГУМОРАЛЬНЫЕ НАРУШЕНИЯ СИСТЕМЫ АДАПТАЦИИ ПРИ ВОЗДЕЙСТВИИ НЕКОТОРЫХ КЛАССОВ ПЕСТИЦИДОВ

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В практике агропромышленного комплекса в последние годы большое значение приобретает комбинированная обработка сельскохозяйственных культур смесью из пестицидов, относящихся к различным химическим классам и обладающих разным типом действия [1]. Этот агротехнический прием позволяет расширить спектр действия пестицидов, сокращает рабочее время и материальные затраты, предотвращает загрязнение окружающей среды.

Применение комбинированных смесей из пестицидов может непосредственно или опосредованно вызывать различные расстройства в организме человека и животных [2]. Известно, что в зависимости от дозы и продолжительности воздействия смесь одних и тех же веществ может оказывать на организм различный по своему характеру эффект [3]. К основным видам комбинированного действия относят эффекты суммирования или аддитивного действия, потенцирования или синергизма и антагонизма применяемых пестицидов [4].

Попадая в кровь, пестициды распределяются в организме неравномерно. Препараты, хорошо растворимые в липоидах и жирах, обычно в больших количествах проникают в нервную ткань и в ряде случаев оказывают преимущественное влияние на центральную нервную систему. Различные органы и ткани неодинаково чувствительны к действию различных пестицидов [5].

В настоящее время особый интерес ученых вызывает нейротоксическое действие синтетических пиретроидных инсектицидов, представителем класса которых является препарат суми-альфа. Показано, что инсектициды имеют низкую токсичность, однако вызывают тяжелые нейротоксические симптомы при кумуляции в нервной системе [6].

Никотин является одним из основных токсичных компонентов табака (табачная пыль). Исследователями установлено [7], что образцы табачной пыли содержат такие макро- и микроэлементы, как кремний, кальций, барий, стронций, магний, титан, олово, никель, медь, цирконий, цинк, хром,