

DENTISTRY

Nazaryan R.S., Iskorostenskaya O.V., Gorenskaya O.V., Volkova N.E

ANALYSIS OF VNTR POLYMORPHISM OF MUC5B GENE IN CONNECTION WITH CERTAIN PHYSICO-CHEMICAL PROPERTIES OF ORAL LIQUID IN CHILDREN WITH DOWN SYNDROME

Kharkiv National Medical University, Ukraine

Abstract: Protective function of oral fluid is evident in maintaining constant saliva volume, moisturizing mucous membranes of the oral cavity, teeth enamel, preventing the defeat of soft and hard tissues of the oral cavity by pathogenic microorganisms. A number of factors, called "barriers of colonization", specifically and nonspecifically manage the process. What matters most is "mucous block", which characterizes the set of mechanical, humoral, nonspecific factors of protecting mucous membranes against microorganisms. Mucin proteins which are the main glycoprotein saliva components affect the creation and selection of biofilm microflora, facilitating or inhibiting the adhesion of microorganisms and maintaining healthy microbial environment in the oral cavity. The dominant mucin of submucosa glands is MUC5B, which is encoded by the same gene, located in a short shoulder of segment 15.5 of chromosome 11. Changes of the basic physical and chemical properties of non-stimulated saliva in children with Down syndrome, namely, reduction of pH level and increasing oral fluid viscosity, is certainly an important prerequisite for formation of cariogenic situation.

KeyWords: Down syndrome, MUC5B, oral cavity, saliva.



INTRODUCTION

The study of molecular-genetic bases of multifactorial diseases including diseases of the oral cavity refers to one of the challenges of modern genetics. Identification of genetic factors predisposing to the development of the disease has a prognostic value and can be used in presymptomatic diagnosis, that is, before the appearance of any clinical or biochemical disease symptoms. The intensity of pathological processes in children's oral cavity is directly related to the composition and properties of oral liquid [1].

Acid-base status and saliva viscosity indicator are one of the most important indices of oral cavity homeostasis [2; 3].

Changes in physical and chemical properties of oral liquid have a significant impact on the structural properties and stability of oral liquid as a colloidal system, leading to destabilization of its micellar state and causing further changes in organs and tissues of the oral cavity, namely disorder of remineralization process of tooth enamel, the change of a state of periodontal tissues and mucous membranes of the oral cavity [4].

Protective function of oral liquid is evident in maintaining frequent volume of saliva, moisturizing mucous membranes of the oral cavity, tooth enamel. All this is essential for keeping the oral cavity organs in a functionally active state, as well as for prevention of soft and hard tissues of the oral cavity from the damage by pathogenic microorganisms. Immunoglobulins, lysozyme, mucin, lactoferrin, nucleases, proteases, myeloperoxidase, salivary peroxidase play the most important role in maintaining this feature [5]. Despite the fact that oral liquid contains only 0.2-0.4% of protein and not more than 2% of other substances, it has high internal structuredness due to the presence of mi-

-----◆-----
Corresponding Author:

Iskorostenskaya O.V., MD, PhD, Professor assistant, Department of Department of Pediatric Dentistry, Pediatric Oral Surgery and Implantology, Kharkiv National Medical University. E-mail: iskorostenskaov@gmail.com

celles based on calcium phosphate. The presence of acid proteins, rich in proline, in mixed saliva, gives it viscosity and ductility [6]. Mucin proteins, which are the main glycoprotein components of saliva, affect the creation and selection of microflora in biofilm, facilitating or preventing from the adhesion of microorganisms and maintaining healthy microbial environment in the oral cavity [6].

Every person has 11 families of mucins encoded by non-allelic genes. Genes' expression is found in the buccal salivary glands, mucosa of respiratory tract and cholecyst. All mucins are glycoproteins with high amount of carbohydrates. Dominant mucin of submucosa glands is MUC5B [7] which is encoded by the homonymous gene, located in a short shoulder of segment 15.5 of chromosome 11.

2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose of this work is to study VNTR polymorphism (variable number of tandem repeats) of MUC5B gene and to search for connection of this gene's polymorphism with level pH and viscosity of oral liquid in children living in Kharkiv. Selected for the study the option of VNTR polymorphism of MUC5B gene is connected with including in the gene similar fragments from 59 base pairs in introne 36 [8]. Analysis of polymorphism associated with the satellite regions of the gene is informative enough, because it allows to identify the diversity of allelic forms of a gene in a population.

2.2 Subjects & Methods

The study was conducted at the University Dental Center of Kharkiv National Medical University. Total number of examined patients was 43 children aged from 2 to 17 years. The main group comprised 9 children with Down syndrome. The control group included 34 children without chromosomal pathology. To compare the main and control groups adequately, we divided the control group into 2 age categories: the first for children from 2 to 8 years (9 patients - control 1) and the second is for children from 9 to 17 years (25 patients - control 2). All the examined children and their parents have been informed of the aim of

the study and the methods to be applied. Parents have given written consent to participate in the study.

Determination of pH in oral liquid. Determination of pH of mixed saliva was carried out with the help of test strip indicator ("SPOFA", The Czech Republic). In patients with Down syndrome we collected non-stimulated oral liquid on glass. Test strips for determining pH were sunk in a drop of oral liquid for 10 seconds, then the color of the test strips was compared with the table from a set [9; 10; 11].

Viscosity determination method (gradation level) of oral liquid [4] implied that thin threads were extruded from saliva accumulated in the sublingual region within 2 minutes with the help of dental forceps. Cutting threads happened at some level which was the basis for detecting four gradations of viscosity test, designated by points from 1 to 4. 1 - sharply negative (cutting threads at the level of the central teeth of the upper jaw or upper lip); 2 - negative (cutting threads at the level of the wings or nose tip); 3 - positive (cutting thread at the brow level); 4 - sharply positive test (cutting threads at the scalp level and above) [12] [10; 11].

To conduct genotyping buccal epithelial cells were used. Selection of material for the study was conducted during dental examination by using sterile disposable urogenital probe in an individual container marked in accordance with the method [13]. DNA was isolated by using a commercial set Diatom™ DNA Prep 100 (Russia) in accordance with the manufacturer's instructions [14]. Typing VNTR polymorphism in introne 36 of MUC5B gene was conducted by using polymerase chain reaction (PCR) with detection of amplified fragments in agarose gel. For amplification such primers were used: MUC5BF - 5'- AGTGTG-CAGTGACTGGCGAG-3' and MUC5BR - 5'- CTAGAGTTGCAGGTGGCAGG-3' [15]. Automatic thermocycler "Tercik" (Russia) and commercial sets of reagents GenPak™ PCR Core (0.5 ml) (Russia) were used for PCR of alleles of MUC5B gene in accordance with the manufacturer's instructions. PCR conditions: denaturation for 3 min at 95°C; 30 cycles consisting of denaturation for 30 s at 95°C, annealing of primers for 30 s at 95°C, elongation for 45 s at 72°C; final elongation for 7 min at 72°C [15]. Detection

of PCR results was conducted by dividing the amplification products in 2% agarose gel at constant voltage 70V within an hour. Commercial sets ELA-50 ("Neogene", Ukraine) were used for electrophoresis. Visualization of the fragments was conducted by processing gel with ethidium bromide and subsequent analysis on transilluminator in ultraviolet light. The size of the fragments was determined in comparison with a molecular weight of pUC19 DNA/Mspl (HpaII) Marker, 23 (Thermo Fisher Scientific Inc.).

Statistical analysis of the results. The difference between the control and the main groups of the alleles in introne 36 of MUC5B gene was established by using the Kraskell-Wallace criterion. The difference between the control and the main groups on quantitative criterion (oral liquid pH level), as well as the dependence of quantitative indication from allele in introne 36 of MUC5B gene was established by using variance analysis (ANOVA). Reliability of differences was assessed by Student's t-test. Statistical processing of data and mathematical analysis was carried out by using BioStat 2008 Professional, frequency of MUC5B gene alleles was calculated by using GenoMprofessional.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

The results of the study on indices of acidity and viscosity of non-stimulated oral liquid in children living in Kharkiv region are shown in Figure 1.

Children with Down syndrome, in comparison with the control group children, were shown to have a positive change in viscosity index of saliva - cutting salivary threads was carried out at the brow or the scalp level. The results of the variance analysis revealed a significant difference of this indicator in the main and the control group 1 ($F = 6.05$; $p < 0.05$ for K1 and $F = 4.58$; $p < 0.05$ for K2). Perhaps this is because children with Down syndrome have a decrease in the secretion of the salivary parotid (Spec Care Dentist).

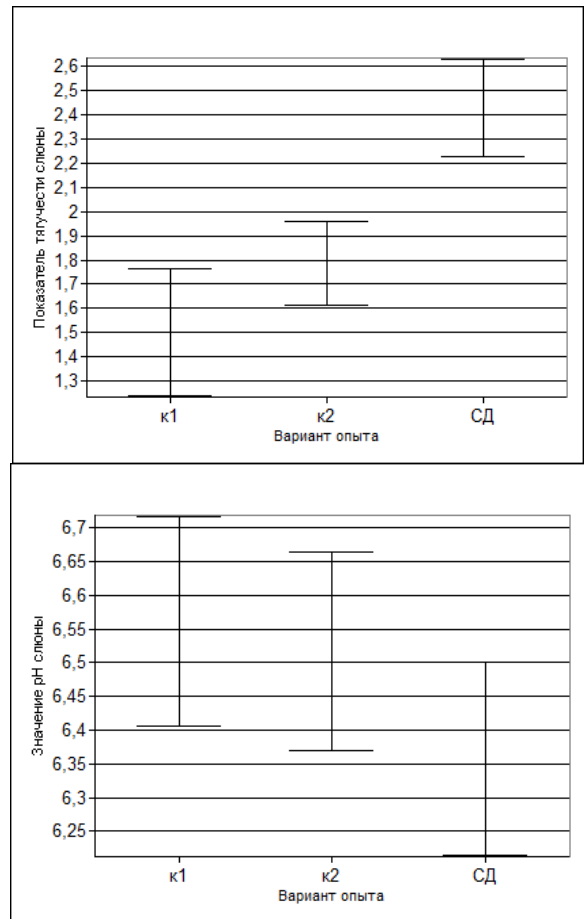


Fig. 1. The differences in level of viscosity and acid-base status of non-stimulated oral liquid in main (DS - children diagnosed with Down syndrome) and control (K1 and K2) groups.

Rheological properties of saliva, which include viscosity and ductility, characterize the overall functional state of the body. This is quite a sensitive indicator, and even short-term and slight chemical and metabolic disorders, accompanying generally somatic pathologies, can change it [16; 17; 18]. The results of our study showed a downward tendency in pH level of oral liquid in children with Down syndrome in comparison with the children of both authors [19]. The variance analysis showed no reliable difference of this indicator in the main and control groups ($F = 0.9$; $p > 0.05$ for K1 and $F = 0.28$; $p > 0.05$ for K2).

Changes of the basic physical and chemical properties of non-stimulated saliva in children with Down syndrome, namely reduction of pH level and increasing oral liquid

viscosity, is certainly an important precondition for the formation of a cariogenic situation.

The main factors of destabilization of the physical and chemical properties of the oral cavity in norm and pathology are meal and metabolic activity of microorganisms. Oral cavity is colonized by representatives of different taxonomic groups of microbes that enter into biochemical, immunological and other interactions with macroorganism and with each other, forming microbiocenosis of the oral cavity [20; 21]. Colonization of conditionally pathogenic microbes of the oral cavity in low resistance of teeth tissues to caries creates conditions for

There is a number of factors called "barriers of colonization", specifically and non-specifically governing this process [22]. The greatest value has the "mucous block" which characterizes the set of mechanical, humoral, non-specific factors of mucosal protection against microorganisms [23].

The main protective protein that stabilizes minerals in saliva, supporting its micellar composition, is mucin which is encoded by MUC5B gene. Gene MUC5B is included in 4-genic cluster located in segment p 15.5 of short shoulder of chromosome 11. Gene in a human genome has 40 triphosphopyridine nucleotides, their encoding sequence having 10713 nucleotides [24].

The results of the genetic analysis have showed that in the population of Kharkiv there are 8 types of alleles differing from each other by the number of tandem repeats in introne 36 of MUC5B gene. Among the examined the most frequent ones have been homozygotes (21 people - 48.8% of all the examined), their alleles having 2, 8, 5, 7 and 6 repeats, respectively, 10, 4, 3, 3 and 1 people. The less presented ones have been heterozygotes - 8 people (18.6% of all the examined). The most commonly detected individuals are ones with options 7/9 (3 people), also there are combinations of alleles of 3/7, 8/9, 2/7, 2/3, 6/9 (1 examined).

Checking by Kraskell-Wallace criterion has not shown significant differences in the frequency of alleles of MUC5B gene with different number of repeats in the control and main group ($p = 0.1821$). The results of the study of 14

examined (32.6%) have shown the presence of three allelic MUC5B genes in genotype. In 3-allelic genotype combinations there are dominant options of alleles with three (25.5%) and eight (21.6%) repeats. Extreme options - 2 and 9 repeats - by 3.9%, respectively, are less presented.

It is interesting to note that the control and main groups are significantly different in the frequency of individuals with VNTR alleles - 22.2% and 37.5%, respectively. The presence of three alleles in one genotype can be explained by the high frequency of recombination, which demonstrates, in our opinion, an increased level of genome instability in the main group of children (diagnosed with Down syndrome). It has been previously shown that trisomy of chromosome 21 affects the transcription of genes of the chromosome involved in numerical chromosomal anomaly and the frequency of unequal X-inactivation [25].

Basic recombination mechanisms may be unequal chromosomal crossing-over, gene conversion and exchange of sister chromatids. Increased frequency of recombinations is directly connected with the epigenetic mechanism and may be the result of non-methylated cytosine in satellite repeats. It is expected that methylation prevents from undesired recombinations between homologous satellites in shifted positions and helps stabilize the tandemly located units in the cell nucleus [26]. DNA methylation is a stable epigenetic modification changing the pattern of gene expression. However, throughout the life of the individual DNA methylation profile changes can occur. These changes are often connected with any pathological process, such as oncogenic transformation, cell aging or hereditary diseases [27]. This is confirmed by the results obtained in our work - children with Down syndrome have an increased frequency of 3-allelic combinations of MUC5B gene.

It is obvious that genetic instability in children diagnosed with Down syndrome, shown by us on the example of MUC5B gene, is the reason causing the change of physical and chemical properties of oral liquid. Thus, the increase in a viscosity indicator of oral liquid in children with Down syndrome, as shown in our work, can

be explained by the increase in the concentration of mucin in saliva.

The analysis of VNTR polymorphism in introne 36 of MUC5B gene has shown no significant association with acidity and viscosity of oral liquid of the examined children. The results of the variance analysis (ANOVA) when identifying the contribution of each of the alleles are shown in Table 1.

Table 1.

The contribution of alleles with different numbers of repeats in introne 36 of MUC5B gene in indices of acidity and viscosity of oral liquid (ANOVA results)

VNTR polymorphism in introne 36 of MUC5B gene	Salivary viscosity			Salivary pH		
	MS	F	Significance level	MS	F	Significance level
2 repeats	0.91	0.34	0.52	0.35	0.83	0.36
3 repeats	0.05	0.07	0.79	0.39	0.92	0.33
4 repeats	0.12	0.15	0.69	0.15	0.39	0.53
5 repeats	0.17	0.22	0.64	0.81	2.01	0.16
6 repeats	0.32	0.42	0.52	0.04	0.10	0.75
7 repeats	1.36	1.89	0.18	0.02	0.04	0.84
8 repeats	0.14	0.18	0.67	0.36	0.90	0.34
9 repeats	0.02	0.02	0.88	0.15	0.36	0.55

CONCLUSIONS

Thus, the results have led to the following conclusions:

1. Analysis of acid-base balance of the oral cavity has revealed that children with Down syndrome, compared to the children of control age groups, have a decrease in oral liquid pH level ($F = 0.9$; $p > 0.05$ for K1 and $F = 0.28$; $p > 0.05$ to K2) and an increase in viscosity of non-stimulated saliva ($F = 6.05$; $p < 0,05$ for K1 and $F = 4,58$; $p < 0,05$ for K2).

2. The results of VNTR polymorphism in introne 36 of MUC5B gene have detected that in the children population of Kharkiv there are alleles with two (0.379), seven (0.190) and eight (0.155) repeats. 32.6% of the examined have 3-allelic combinations of MUC5B gene in genotype. The control and main groups differ in frequency of individuals with VNTR alleles - 22.2% and 37.5%, respectively.

3. Significant associations of VNTR polymorphism in introne 36 of MUC5B gene with acidity of the oral cavity and saliva viscosity indices of the examined children are not shown.

REFERENCES

1. Kovach I.V., Pivovarov M.Yu., Verbitskaya A.V. (2013) *Izmenenie fizicheskikh svoystv rotovoy zhidkosti u detey s rashchelinami tverdogo i miagkogo neba v dinamike* [Change of physical properties of oral liquid in children with clefting hard and soft palate in dynamics]. *Sovremennaya stomatologiya*, no 3, pp. 68-73.
2. Skatova E.A., Makeev M.K., Shakariants A.A. (2010) *Prakticheskie aspekty opredeleniya riska razvitiya kariesa* [Practical aspects of determining the risk of caries development]. *Problemy stomatologii*, no 3, pp. 41-46.
3. Den'ga O.V., Den'ga E.M., Levitskiy A.P. (1995) *Informativnost' pH-testa sliuny pri provedenii sanatsionno-profilakticheskikh meropriyatiyakh* [Informative value of the pH-test of saliva when conducting sanitation and preventive measures]. *Visnyk stomatologii*, no 1, pp. 42-45.
4. Bondarik E. A., Gorodetskaya O.S., Beliasova L.V. (2004) *Biokhemieskie i biophysicheskie svoystva rotovoy zhytkosti u patsyentov s vysokim urovnem intensivnosti kariesa zubov* [Biochemical and biophysical properties of oral liquid in patients with a high level of caries intensity]. *Belorusskiy meditsinskiy jurnal*, no 4, pp. 36-38.
5. Gilmiyarova F.N., Radomska V.M., Babichev A.V. (2006) *Analiticheskie podkhody k izucheniyu pokazayey metabolizma v rotovoy zhidkosti* [Analytical approaches to the study of metabolic indicators in oral liquid]. *Moskva: Meditsina*.
6. Marsh, P.D. (2003) *Are dental diseases examples of ecological catastrophes?* *Microbiology*, 149, 279-294.
7. Davies, J.R. [et al.] (2002) *Respiratory tract mucins: structure and expression patterns*. *Novartis Found.*

- Symp., 248, 76-88.
8. Desseyn, J.-L., Rousseau, K., Laine, A. (1999) Fifty-nine bp repeat polymorphism in the uncommon intron 36 of the human mucin gene MUC5B. *Electrophoresis*, 20, 493-496.
 9. Kulygina V.N., Ali, A. M.M. (2014) Issledovanie skorosti sliunootdeleniya i sostoyaniya kislotno-shchelochnogo ravnovesiya v rotovoy polosti u lits molodogo vozrasta s vospalitel'nymi zabolovaniyami tkaney parodonta [The study of speed of salivation and condition of acid-base balance in the oral cavity in young people with inflammatory diseases of periodontal tissues]. *Jurnal vushnykh, nosovykh i gorlovykh khvorob*, no 3, pp. 69-73.
 10. Domeniuk D.A. Risovanniy I., Risovannaya O.N. (2013) Vliyanie bazisnykh materialov, ispol'zuemykh v syiomochnykh ortodonticheskoy apparature u detey na adaptatsionnye mekhanizmy [Influence of the basic materials used in removable orthodontic equipment in children on adaptation mechanisms]. *Kubanskiy nauchniy meditsinskiy vestnik*, no 6 (141), pp. 93-98.
 11. Dedova L.N., Kandrukevich O. V. (2013) Karies kornia: klinika, diagnostika, lechenie: Uchebno-metodicheskoe posobie [Root caries: clinical picture, diagnosis, treatment: Manual]. Minsk: BGMU.
 12. Redinova T.L., Pozdeev A.R. (1994) Klinicheskie metody issledovaniya sliuny pri kariесе zubov: Metodologicheskie rekomendatsii dlya subordinatov, internov i vrachey-stomatologov [Clinical research methods of saliva in dental caries: Methodological recommendations for subordinators, interns and dentists]. Izhevsk: Meditsina.
 13. Claire, M., Isabelle, S., Jacqueline, C. [et al.] (2005) Collection of Human Genomic DNA From Buccal Cells for Genetics Studies: Comparison Between Cytobrush, Mouthwash, and Treated Card. *Journal of Biomedicine and Biotechnology*, 3, 291-296.
 14. Galart Diagnosticum (2014). Diatom™ DNA Prep 100. Nabor reagentov dlia vydileniya DNK iz razlichnogo biologicheskogo materiala: Instruksiya [Diatom™ DNA Prep 100. The set of reagents for sectering DNA from different biological material: Instruction]. Retrieved from http://www.galartdiag.ru/files/diatom_dna_prep_100.pdf
 15. Vinall, L.E., Fowler, J.C., Jones, A.L. [et al.] (2000) Polymorphism of human mucin genes in chest disease, possible significance of MUC2. *Am. J. Respir. Cell. Mol. Biol.*, 23, 678-686.
 16. Yarov Yu.Yu. (2013) Sravnitel'niy analiz reologicheskikh parametrov rotovoy zhitzkosti pri razlichnoy stepeni porazheniya parodonta [Comparative analysis of rheological parameters of oral liquid with various degree of periodontal lesion]. *Visnyk stomatologii*, no 1, pp. 43-47.
 17. Afanasyeva L.R. (2000) Funktsional'nye svoystva i sostav rotovoy zhytkosti u detey s narusheniem razvitiya intellekta [Functional properties and structure of oral liquid in children with impaired development of intelligence]. *Sovremennaya stomatologiya*, no 3, pp. 24-26.
 18. Moiseeva M.V., Shakirova R.R., Nikolaeva E.V. (2015) Korrektsiya reologicheskikh svoystv sliuny u detey s patologiyey zheludochno-kishechnogo trakta [Correction of rheological properties of saliva in children with pathology of the gastrointestinal tract]. *Aspirantskiy vestnik Povolzhya*, no 5-6, pp. 294-298.
 19. Davidovich, E. [et al.] (2010) A comparison of the sialochemistry, oral pH, and oral health status of Down syndrome children to healthy children. *Int. J. Paediatr. Dent.*, 20, 235-241.
 20. Zakharov A.A., Ilyina N.A. (2007) Analiz mikroflory rotovoy polosti obsledovannykh liudey s razlichnymi zabolovaniyami [Analysis of microflora in oral cavity of the examined people with various diseases]. *Uspekhi sovremennogo yestestvoznaniya*, no 12-3, pp. 141-143.
 21. Simonova E.V., Ponomariova O.A. (2008) Rol' normal'noy mikroflory v podderzhanii zdorov'ya cheloveka [Role of normal microflora in maintaining human health]. *Sibirskiy meditsinskiy jurnal*, no 8, pp. 20-25.
 22. Akhremenko Ja.A. (2008) Mikrobiologiya polosti rta:

- Uchebnoe posobie dlya studentov stomatologicheskikh fakul'tetov [Oral microbiology: Tutorial for students of dental faculties]. Yakutsk: Izdatel'stvo Yakutskogo Universiteta.
23. Dobren'kov D.S. (2014) Kharakteristika biotsenoticheskikh otnosheniy bakterian'nykh soobshchestv polosti rta i mikroekologicheskoe obosnovanie printsipov biokorreksii [Characteristics of biocenotic relations of bacterial communities of the oral cavity and microecological basis of biocorrection principles]. Ph.D. Dissertation, UGMU, Volgograd.
24. Desseyn J.-L. (1997) Human mucin gene MUC5B, the 10.7-kb large central exon encodes various alternate subdomains resulting in a super-repeat. Structural evidence for a 11p15.5 gene. *J. Biol. Chem.*, 272 (6), 3168-3178.
25. Vorsanova, S.G., Yurov, Y.B., Beresheva, A.K. [et al.] (2003) Aliphoid DNA variation and non-disjunction in Down syndrome: fluorescence in situ hybridization and cytogenetic. *Balkan J Med Genet*, 6 (3-4), 81-86.
26. Deichman A.M. (2015) Povtory, nestabil'nost' genoma i onkogenez. Vozmozhny biosintez oligonukleotidov vne genoma [Repeats, genome instability and oncogenesis. Possible biosynthesis of oligonucleotides out of genome]. Moskva: Mir nauki.
27. Kabanov I. N., Tishchenko L. I. (2014) Izmenenie metilirovaniya DNK povtoriyayushchikhsia posledovately I odnokopiynykh genov pri onkologicheskikh i nekotorykh drugikh zabolevaniy cheloveka [Changing the methylation of DNA of repeated sequences and single-copy genes in cancer and some other diseases]. *Vestnik Sant Peterburgskogo Universiteta (Seria 3: Physiologiya, Biokhimiya, Biofizika)*, no 3, pp. 62-83.

Received: 03-Aug. - 2017

Accepted: 12-Nov. - 2017

