

Review Article

ROLE OF COL1A1 AND G2046T GENES IN UZBEKS WITH JUVENILE  
DYSMENORRHEA IN THE PRESENCE OF CRITERIA FOR UNDIFFERENTIATED  
CONNECTIVE TISSUE DYSPLASIA

<sup>1</sup>Oltinoy Yakubova, <sup>2</sup>Farida Ayupova, <sup>3</sup>Zainitdin Kamalov, <sup>4</sup>Khabibahon Negmatshaeva, <sup>5</sup>Dilfuzahon Mamarasulova

Andijan Medical Institute, Uzbekistan. E-mail address: [article\\_01@mail.ru](mailto:article_01@mail.ru)

Received: 15.11.2019

Revised: 16.12.2019

Accepted: 26.01.2020

Abstract

Clinical and genetic examination of 118 girls was conducted, with signs of connective tissue dysplasia (CTD) - 64 and without it - 54, the control group consisted of 68 healthy girls with normal menstruation. The basis for the diagnosis of dysmenorrhea was a complaint of painful menstruation. An analysis of the distribution of allelic variants of the COL1A1 G2046T gene showed that in the group of patients with juvenile disease accompanied by CTD significantly higher compared with the control group of practically healthy individuals, only a tendency toward the reliability of alleles was observed, but they did not reach true significance. The study of the association of the COL1A1 G2046T genotype showed a significant increase in the mutant TT genotype in the group of girls with juvenile disease with CTD, compared with practically healthy individuals. Therefore, the single nucleotide variant of COL1A1 G2046T plays a role in the pathogenesis of the development of juvenile disease accompanied by CTD. This is indirectly confirmed by the indicators of oxyproline, an increase in which was significant both in comparison with practically healthy individuals and in comparison with a group of patients with juvenile disease without signs of CTD, suggests that this polymorphism is only one of the polymorphisms involved in the pathogenesis of the studied pathology. Based on the foregoing, we can conclude that the occurrence of the mutant allele of the estrogen receptor gene - alpha (single nucleotide replacement of guanine with adenine in rs2228480) is 1.9 times higher in the group of girls with juvenile dysfunction with CTD than in the group of juvenile dysfunction with CTD ( $\chi^2 = 4.515$ ;  $p = 0.03$ ). A significant difference was also found in the frequency of occurrence of type I collagen gene polymorphism (single nucleotide replacement of guanine with thymine in rs1800012) was found in girls with juvenile disease with CTD only in comparison with a group of healthy individuals ( $\chi^2 = 4.71$ ;  $p = 0.03$ ).

**Key words:** primary dysmenorrhea, juvenile dysmenorrhea, connective tissue dysplasia, estrogen receptor alpha (ESR1), COL1A1 G2046T genes.

© 2019 by Advance Scientific Research. This is an open-access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)  
DOI: <http://dx.doi.org/10.31838/jcr.07.02.75>

INTRODUCTION

Around the world, one of the factors that worsen the quality of life of girls and adolescent girls is considered to be a pain syndrome accompanying the physiological process - menstruation [13,22,26]. According to the WHO, the prevalence of menstrual pain in the structure of adolescent gynecological pathology is extremely high, while about 15% of them characterize menstrual pain as painful [1,2,21,23]. Juvenile dysmenorrhea (JD) - painful menstruation in girls under 18 in the absence of pelvic pathology is a common and often depleting gynecological suffering, regardless of age or nationality [15,19,25]. Despite the high prevalence, primary dysmenorrhea in girls is often poorly diagnosed and even ignored by medical professionals and the girls themselves and their mothers, who can accept painful menstruation as a normal part of the menstrual cycle [7,18,20].

JD is a signal of disorders that have developed in systems that provide and control the process of endometrial rejection [4]. When a pathological situation arises in the body of a growing female body, the formation of pathological conditions of organs and tissues in the form of undifferentiated connective tissue dysplasia (CTD) occurs [6,12]. The main component of connective tissue is collagen fibers, and oxyproline is a biochemical marker of its breakdown [5]. Connective tissue is constantly updated, undergoes restructuring in response to stress and damage. The intensity of biosynthesis of collagen fibroblasts depends on many factors: hereditary, hormonal, metabolic [9]. When studying CTD, the question arises about the possible cause of changes in connective tissue based on a genetic predisposition. As you know, the manifestation of a

particular disease is often caused by a combination of certain allelic variants of genes, polymorphisms in the genotype of the growing organism, and polymorphisms that form a certain hereditary background, which can be realized when the pathological genotype interacts with environmental factors.

In a number of studies, some hypotheses have been found below, which are relevant to the present. In these works, morphological changes characteristic of NDCT and changes in genes encoding the synthesis and spatial organization of collagen, as well as gene defects of enzymes, cofactors, and steroid hormones, leading to changes in the architectonics of connective tissue, were revealed. The influence of the medium in this case plays the role of triggering factors [10,16]. As you know, collagen is one of the most abundant proteins in the extracellular matrix and in connective tissue. Collagens differ in their position in the tissue and in the function they carry. There are four main types of collagen (I – IV), which include the following genes: collagen I (genes COL1A1, COL1A2) - the main component of bone, which is also present in scars, tendons and cartilage; collagen II (COL2A1 gene) - the main component of cartilage; collagen III (COL3A1 gene) forms reticular fibers that hold the extracellular matrix together; collagen IV (genes COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, COL4A6) forms the basal lamina on which the epithelium rests. [11,24]. It was found that in women with systemic connective tissue insufficiency, a partial decrease in type I collagen in the interstitial substance is observed, which is probably a consequence of a violation of collagen secretion while maintaining its synthesis [17].

Some studies have revealed that all patients with CTD, regardless of the severity of type I and III collagen, had an atypical spatial structure without the formation of pronounced fiber bundles, and then there was a replacement in the ligamentous apparatus of type I and III collagen with type IV collagen, which led to deep violations of the mechanical characteristics and functional insufficiency of the design of the ligament-supporting tissues of the pelvis [8]. There is an opinion that in the presence of a genetic predisposition in the future, especially with the adverse effects of external factors, one or another clinical form of the disease is observed. In order to detect this genetic predisposition, a number of scientists have studied type I collagen receptor gene polymorphism (COL1A1). The gene for the  $\alpha 1$  chain of type I collagen (COL1A1) is located on chromosome 17q21.3-22. The G2046T polymorphism is a point substitution of G at T position 2046, localized in the non-coding region of the gene affecting the binding site of the transcription factor gene alpha-1 collagen type 1 chain. Considering CTD as a result of a defect in collagen genes, much attention is given in the literature to the components responsible for the metabolism of the latter: fibrillogenesis proteins, cross-linking, responsible for the ordered distribution of collagen chains and its remodeling (degradation and proteolysis) [3]. To date, morphological changes characteristic of CTD and changes in genes encoding the synthesis and spatial organization of collagen, as well as gene defects of enzymes, cofactors, and steroid hormones leading to changes in the architectonics of connective tissue have been identified [8,11,24].

The purpose of the study was the study of COL1A1 G2046T polymorphism in girls with juvenile dysmenorrhea depending

on the presence of connective tissue dysplasia in the Uzbek population

#### MATERIAL AND RESEARCH METHODS

Clinical and genetic examination of 118 girls aged 13 to 18 years was conducted, with signs of CTD - 64 and without it - 54, the control group consisted of 68 healthy girls with normal menstruation. The genetic study was carried out by polymerase chain reaction (PCR) using specific primers (NPF Litech, Russia) in an automatic rotor Geene 6000 thermocycler. The determination of free and bound hydroxyproline in urine was carried out according to the method of P. N. Sharaev [4]. The data obtained during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2012 software package.

#### RESULTS OBTAINED AND DISCUSSION

The basis for the diagnosis of dysmenorrhea was a complaint of painful menstruation. Patients to exclude organic pathology i.e. secondary dysmenorrhea, a health survey was performed (ultrasound of the pelvic organs, a smear on the flora, examination by a vertebrologist).

As shown in Table 1, an analysis of the distribution of the allelic variants of the COL1A1 G2046T gene showed that in the group of patients with juvenile disease accompanied by CTD significantly higher compared with the control group of practically healthy individuals, only a tendency toward the reliability of alleles was observed, but they did not reach true significance.

Frequency distribution of alleles and genotypes of the COL1A1 G2046T gene in girls with JD with CTD compared with the control group of healthy individuals

Table 1

Geno type	JD + CTD		Control		OR	$\chi^2$	P
	n=64	%	n=68	%			
G	97	75,78	114	83,82	0,60	2,658	0,1
T	31	24,22	22	16,18	1,66	2,658	0,1
GG	41	64,06	48	70,59	0,74	0,639	0,4
GT	15	23,44	18	26,47	0,85	0,162	0,6
TT	8	12,50	2	2,94	4,71	4,302	0,03

Then, a significant increase in the TT homozygous genotype was observed in the group of girls with juvenile disease and CTD compared with the control group ( $\chi^2 = 4.302$ ,  $p < 0.03$ ,  $OR \geq 4.71$ ). Further, when studying the distribution of the allelic variants of the COL1A1 G2046T gene in the group of patients with juvenile disease accompanied by CTD compared with the group of girls with JD without CTD, it was found that there were

no significant differences in the frequencies of alleles in these groups (Table 2).

Frequency distribution of alleles and genotypes of the COL1A1 G2046T gene in girls with JD with CTD compared with the control group with JD without CTD

Table 2

Genot ype	JD + CTD		JD without CTD		OR	$\chi^2$	P
	n=64	%	n=54	%			
G	97	75,78	88	81,48	0,71	1,124	0,2
T	31	24,22	20	18,52	1,41	1,124	0,2
GG	41	64,06	37	68,52	0,82	0,26	0,6
GT	15	23,44	14	25,93	0,87	0,09	0,7
TT	8	12,50	3	5,56	2,43	1,671	0,2

At the next stage, it was decided to analyze the distribution of the frequency of occurrence of allelic variants and COL1A1 G2046T genotypes in the group of girls with juvenile disease without CTD compared to practically healthy individuals in the population control.

Frequency distribution of alleles and genotypes of the COL1A1 G2046T gene in girls with JD without CTD compared with the control group of healthy individuals

Table 3

genotype	JD without CTD		Control		OR	$\chi^2$	P
	n=54	%	n=68	%			
G	88	66,67	114	83,82	0,85	0,23	0,6
T	20	18,52	22	16,18	1,18		
GG	37	68,52	48	70,59	0,91	0,06	0,8
GT	14	25,93	18	26,47	0,97	0,005	1
TT	3	5,56	2	2,94	1,94	0,523	0,4

As can be seen from table 3, in the course of the analysis of these groups, there was no significant difference either for allelic variants or for genotypes.

Thus, when studying the association of the COL1A1 G2046T genotype, a significant increase in the mutant TT genotype was

revealed in the group of girls with JD with CTD, compared with practically healthy individuals.

The level of oxyproline in daily urine ( $\mu\text{mol} / \text{day}$ ) in girls with JD, depending on the presence of CTD criteria,  $M \pm m$

Table 4

Groups	Oxyproline content, $\mu\text{mol} / \text{day}$		
	free	Peptide-linked	Protein-linked
Almost healthy, n=25	18,4 $\pm$ 1,34	155,7 $\pm$ 13,6	8,4 $\pm$ 0,63
JD without CTD			
light, n=10	18,81 $\pm$ 0,30	156,63 $\pm$ 0,16	8,33 $\pm$ 0,26
average, n=31	18,42 $\pm$ 0,27	155,81 $\pm$ 0,24	8,32 $\pm$ 0,23
heavy, n=15	17,82 $\pm$ 0,35	156,33 $\pm$ 0,37	8,11 $\pm$ 0,35
JD with CTD			
light, n=24	26,02 $\pm$ 0,96 <sup>a,6</sup>	163,64 $\pm$ 0,97 <sup>a,6</sup>	8,45 $\pm$ 0,55
average, n=100	34,54 $\pm$ 1,07 <sup>a,6</sup>	167,33 $\pm$ 0,92 <sup>a,6</sup>	8,20 $\pm$ 0,63
heavy, n=50	57,83 $\pm$ 0,88 <sup>a,6</sup>	171,06 $\pm$ 0,97 <sup>a,6</sup>	8,82 $\pm$ 1,51

Note: a - differences relative to data from a group of healthy girls are significant, b - differences relative to data from a group of girls with JD without CTD are significant ( $P < 0.05$ ).

This, possibly, suggests that the single nucleotide variant of COL1A1 G2046T plays a role in the pathogenesis of the development of JD accompanied by CTD. This is indirectly confirmed by oxyproline indicators (Table 4), but taking into account the fact that the increase in the level of this indicator was significant both in comparison with practically healthy individuals and in comparison with a group of patients with JD without signs of CTD, we can assume that This polymorphism is only one of the polymorphisms involved in the pathogenesis of the studied pathology.

### CONCLUSIONS

1. the detectability of oxyprolineuria in girls with NDCT in juvenile dysmenorrhea indicates a violation of the state of collagen in the connective tissue, which is part of the ligaments of the pelvic organs
2. the occurrence of the mutant allele of the estrogen receptor gene - alpha (single nucleotide replacement of guanine with adenine in rs2228480) is 1.9 times higher in the group of girls with juvenile disease with CTD than in the group of juvenile without CTD ( $\chi^2 = 4.515$ ;  $p = 0.03$ ).
3. A significant difference was found in the frequency of occurrence of type I collagen gene polymorphism (single nucleotide replacement of guanine with thymine in rs1800012) was found in girls with juvenile disease with CTD only in comparison with a group of healthy individuals ( $\chi^2 = 4.71$ ;  $p = 0.03$ )

### REFERENCES:

1. Gynecology. National leadership. GEOTAR "Media". Association of Medical Societies for Quality. 2011.- S. 428-437

2. Pediatric and adolescent gynecology. Uvarova E.V. - Moscow, 2009. -- S. 301-318.
3. Gromova O.A. The multifaceted role of macro- and microelements in the construction of bone tissue / Gromova OA, Torshin I.Yu., Limanova OA // Gynecology. - 2014. - T. 16. - No. 2. - S. 50-56.
4. Gynecological endocrinology and reproductive medicine. Michel von Wolff, Peter Stute per. with him. Under the general ed.doc.med.science prof.E.N. Andreeva Moscow MED press-inform. 2018 P.96-101
5. Lukina TS. Assessment of the oxyproline marker and its significance in women with undifferentiated connective tissue dysplasia. Modern medicine: current issues No. 6-7 (41). - 2015. - S. 27-31
6. The national recommendations of the Russian Scientific Medical Society of Therapists on the diagnosis, treatment and rehabilitation of patients with connective tissue dysplasia, developed by a group of specialists in the "Connective Tissue Dysplasia" section of the RNMOT, were approved at the 10th National Congress of Therapists on October 14-16, 2015. Medical Bulletin of the North Caucasus 2016. Vol. 11 No. 1 - S. 247-248.
7. Ozhogina E.V., Moses V.G. Quality of life for women with primary dysmenorrhea. // Questions of gynecology, obstetrics and perinatology. - 2016. - No. 1. - S. 42-44
8. Optimization of the choice of surgical treatment method in patients with complicated forms of genital prolapse / S.N. Buyanova, N.A. Schukina, M.A. Chechnev [et al.] // Russian Bulletin of the Obstetrician-Gynecologist. - 2012. - No. 6. - S87-91.
9. Orlova V.S., Trushina O.V., Kalashnikova I.V. Connective tissue dysplasia is a factor aggravating menstrual pain in adolescence // Journal of Obstetrics and Women's Diseases. - 2018. -- T.67. - No. 1. - S. 38-46.
10. Bubbles, V.P. Pathological anatomy of the human genome / V.P. Puzyrev, V.A. Stepanov. - Novosibirsk: Science. Sib.

- The enterprise of the Russian Academy of Sciences, 1997.  
- S. 22.
11. A systematic analysis of the molecular physiological effects of the synergistic effect of iron, manganese and copper on connective tissue / N.V. Kerimkulova, I.Yu. Torshin [et al.] // *Reproductive endocrinology*. - 2013. - No. 4 (12). - S. 101-110].
  12. Trushina OV, Orlova V.S., Kalashnikova I.V. et al. Physical development and somatic background of teenage girls suffering from dysmenorrhea combined with connective tissue dysplasia // *Scientific Sheets. Series Medicine. Pharmacy*. 2018. Volume 41. No. 1. P.36-45
  13. Seagull G.V., Lomachuk B.O., Kucherenko O. M. Suchly look at the etiology of primary dysmenorrhea. (looking at literature) *Actual nutrition of pediatrics, obstetrics and gynecologists. Obstetrics and gynecology*. - 2015. No. 2. - S. 183 - 188.
  14. Sharaev PN. *Biochemical methods for the analysis of indicators of exchange of biopolymers*. - Izhevsk, 1990. - S. 4-5.
  15. Abaraogu UO, Igwe SE, Tabansi-Ochiogu CS, Duru DO. A Systematic Review and Meta-Analysis of the Efficacy of Manipulative Therapy in Women with Primary Dysmenorrhea. *Explore (NY)*. 2017 Nov - Dec;13(6):386-392. doi: 10.1016/j.explore.2017.08.001. Epub 2017 Aug 30.
  16. Anderson A. Resent advances in the genetics of allergy and asthma / A. Anderson, W.O.C.M. Cookson // *Mol. Med. Today*. - 1999. - V. 5. - P. 264-273.
  17. Blackstock CD, Higashi Y, Sukhanov S, et al. Insulin-like growth factor-1 increases synthesis of collagen type I via induction of the mRNA-binding protein LARP6 expression and binding to the 5' stem-loop of COL1a1 and COL1a2 mRNA. *J Biol Chem*. 2014;289(11):7264-7274. doi:10.1074/jbc.M113.518951
  18. Chen CX, Shieh C, Draucker CB, Carpenter JS. Reasons women do not seek health care for dysmenorrhea. *J Clin Nurs*. 2018 Jan;27(1-2):e301-e308. doi: 10.1111/jocn.13946. Epub 2017 Sep 7.
  19. De Sanctis V, Soliman AT, Elsedfy H, Soliman NA, Soliman R, El Kholy M. Dysmenorrhea in adolescents and young adults: a review in different country. *Acta Biomed*. 2017 Jan 16;87(3):233-246.
  20. Feng X, Wang X. Comparison of the efficacy and safety of non-steroidal anti-inflammatory drugs for patients with primary dysmenorrhea: A network meta-analysis. *Mol Pain*. 2018 Jan-Dec;14:1744806918770320. doi: 10.1177/1744806918770320. Epub 2018 Mar 27.
  21. Gultia, Edmund Joseph, et al. "Ginger root decoction as pain reliever among adolescents with primary dysmenorrhea." *Asian Journal of Health* 3 (2013):1+ Academic OneFile. Web. 25 May 2016
  22. Iacovides S, Avidon I, Baker F. C. Women with dysmenorrhoea are hypersensitive to experimentally induced forearm ischaemia during painful menstruation and during the pain-free follicular phase // *Eur J Pain*. - 2015. - 19:797-804.
  23. Jo J, Leem J, Lee JM, Park KS. Herbal medicine (Hyeolbuchukeo-tang or Xuefu Zhuyu decoction) for treating primarydysmenorrhoea: protocol for a systematic review of randomised controlled trials. *BMJ Open*. 2017 Jun 15;7(6):e015056. doi: 10.1136/bmjopen-2016-015056.
  24. Mann V, Hobson EE, Li B, et al. A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J Clin Invest*. 2001;107(7):899-907. doi:10.1172/JCI10347
  25. Pattanittum P, Kunyanone N, Brown J, Sangkomkhamhang US, Barnes J, Seyfoddin V, Marjoribanks J. Dietary supplements for dysmenorrhoea. *Cochrane Database Syst Rev*. 2016 Mar 22;3:CD002124. doi: 10.1002/14651858.CD002124.pub2.
  26. Woosley J. A., Lichstein K. L. Dysmenorrhea, the menstrual cycle, and sleep // *Behav. Med*. - 2014. - Vol. 40. - P. 14-21.