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Research Article

Polymorphisms in Genes Coding for Transforming Growth Factor ß1 and Matrix Metalloproteinase 9 as a Genetic Predictors of Cervical Incompetence

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Abstract

Introduction: Undifferentiated connective tissue dysplasia is widespread condition among reproductive age women (from 53% to 68.8%). This makes it actual to study the role of pathological connective tissue metabolism in patients with this hereditary syndrome in the development of cervical insufficiency (CI).

Purpose: The aim of this study was to assess the association between single nucleotide polymorphisms (SNPs) *A-8202G (rs11697325) MMP9* gene, *Arg25Pro (rs1800471) TGF*β*1* gene and cervical insufficiency in patients with undifferentiated connective tissue dysplasia.

Methods: A "case-control" study was carried out. Medical, obstetric histories and blood were obtained from 71 women at 22 - 24 weeks of singleton pregnancy with (n = 36) and those without (n = 35) undifferentiated connective tissue dysplasia and CI. Samples were analyzed by using an allele-specific polymerase chain reaction assay for variants in two genes, the MMP9 and *TGFβ1*, encoding connective tissue metabolism. Serum levels of MMP9 and TGFβ1 was determined by enzyme immunoassay (ELISA).

Results and Discussion: Two genotypes were significantly associated with cervical insufficiency compared with controls: homozygous carriers of the **MMP***9-8202G* allele (genotype G/G) (OR = 4.00, 95% CI 1.23-12.98, p = 0.02) and carriers of *Arg/Pro* genotype of the *TGF***β***1* (OR = 3.75, 95% CI 1.88-7.47, p = 0.0001). Carriers of these genotypes had significantly higher serum levels encoding proteins.

Conclusion: *GG* genotype of the *A-8202G* polymorphism **MMP***9* gene and *ArgPro* genotype of the *Arg25Pro* polymorphism *TGF***β***1* gene are associated with a high risk of cervical insufficiency in patients with undifferentiated connective tissue dysplasia.

***Keywords**: Single Nucleotide Polymorphism (SNP); Cervical Incompetence (Insufficiency); Undifferentiated Connective Tissue Dysplasia; Transforming Growth Factor β1 Gene (TGFβ1); Matrix Metallopeptidase 9 (MMP9)*

Introduction

In recent years widespread studies were devoted to the identification of associations of polymorphic variants of genes with a certain disease. This interest of the scientific community can be explained: the determination of genetic predisposition will not only clarify the pathogenesis of the disease, but also develop optimal approaches to its treatment, taking into account the individuality of each patient. The active development of molecular biological and biochemical sciences contributes to the revision of the traditional understanding, including about cervical insufficiency (CI), which remains an urgent problem of modern obstetrics, and is one of the main causes of late

reproductive losses: at about 15 - 40% of late spontaneous miscarriages and 30 - 35% of spontaneous premature birth [1,2]. Researchers point to a link between connective tissue dysplasia and the development of CI, the negative influence of undifferentiated connective tissue dysplasia (CTD) on the locking function of the cervix during pregnancy, a higher frequency of CI in pregnant women with this syndrome, as well as a genetic predisposition to the development of this complication of pregnancy: a third of pregnant women with predisposition have first-line relatives with the same complication of gestation [3]. The cervix is 85% composed of type I collagen. With CI the content of connective tissue in the cervix decreases to 40%, which leads to its early shortening, softening, and the development of functional insufficiency.

Histomorphology studies of cervical tissue in non-pregnant women with CI revealed a lower concentration of collagen and its metabolite - hydroxyproline in comparison with similar indicators of patients without CI in the anamnesis [4-6]. Thus, the pathological metabolism of connective tissue is one of the key factors in the formation of cervical incompetence during pregnancy [7-9].

The importance of genetic factors in the implementation of CI is also confirmed by the results of studies demonstrating that in women with differentiated forms of connective tissue dysplasia (Ellers-Danlo, Stickler, Marfan syndromes), the frequency of premature births associated with CI ranges from 22 to 35% of the total number of births, and up to 40% of them are associated with premature rupture of the fetal membranes [11]. Also, in African women, the risk of developing CI is almost 3 times higher compared to Caucasians (OR = 2.89, 95% CI 2.13 - 3.92) [10].

The prevalence of undifferentiated forms of dysplasia among women of reproductive age is high (from 53% to 68.8%) [12], therefore, the study of CI from this point of view is relevant.

Connective tissue dysplasia refers to hereditary connective tissue diseases. The reason for this condition is the point mutations of the genes encoding the synthesis and metabolism of connective tissue. Due to mutations, there is a violation of the processes of formation and maturation of the spatial structure of collagen and elastin fibers, as a result, there is a decrease in the resistance of these fibers to mechanical loads, acceleration of disorganization and degradation.

The genes encoding the metabolism of connective tissue are the most promising for study. Genes encoding the production of matrix metalloproteinase 9 (MMP9) and transforming growth factor beta 1 (TGFß1) were selected as candidate genes in our study.

MMR 9, or gelatinase B, belongs to the second subfamily of zinc-dependent endopeptidases. The substrate for MMR9 is denatured type I collagen, native collagens of types IV, V, entactin connecting laminin and type IV collagen, which is the main component of the basement membrane of many fibrous organs, including the cervix. As a result of increased activity of MMP 9, massive apoptosis and accelerated cleavage of cells and connective tissue elements from the basement membrane occur, which leads to a rapid decrease in its strength characteristics. A number of researchers have noted high serum concentrations of MMR 9 in preterm birth, as well as the direct involvement of this enzyme in premature rupture of fetal membranes [14,15].

TGFß1 participates in the remodeling of connective tissue by influencing the synthesis of extracellular matrix proteins (collagens of types I, III and fibronectin).It is known that the increased activity of TGFß1 is one of the key factors in the development of such pathological processes in Marfan syndrome as aneurysm and aortic dissection, joint hypermobility, myxomatous changes in atrioventricular valves. TGFß1 increases the production of MMR9 in cells of various types, through a process involving the synthesis of a protein that increases the stability of MMR9 mRNA. On the other hand, the enlarged MMR9, on the contrary, it is capable of splitting latent TGFß1, leading to the activation of the latter according to the feedback principle [16].

Purpose of the Study

The aim of this study was to assess the association between single nucleotide polymorphisms (SNPs) *A-8202G (rs11697325) MMP9* gene, *Arg25Pro (rs1800471) TGF*β*1* gene and cervical insufficiency in patients with undifferentiated connective tissue dysplasia.

Methods

A "case-control" study was carried out. Medical, obstetric histories and blood were obtained from 71 women at 22-24 weeks of singleton pregnancy with (n = 36) and those without (n = 35) undifferentiated connective tissue dysplasia and CI. Samples were analyzed by using an allele-specific polymerase chain reaction assay for variants in two genes, the MMP9 and *TGFβ1*, encoding connective tissue metabolism. Serum levels of MMP9 and TGFβ1 was determined by enzyme immunoassay (ELISA).

The study did not include patients with pregnancy resulting from the use of assisted reproductive technologies, multiple pregnancies, decompensation of obstetric and extragenital pathology, fetal malformations, syndromic forms of connective tissue dysplasia.

The study was performed in accordance with the standards of good clinical practice and the principles of the Helsinki Declaration. The protocol of the study was approved by the Ethics Committee of the Educational Institution "Vitebsk State Medical University".

The examination of pregnant women included the study of somatic and obstetric-gynecological anamnesis, anthropometry (measurement of height, body weight, determination of body mass index), assessment of the severity of phenotypic markers of CTD (according to the ranked scale of evaluation of CTD in pregnant women Kerimkulova N.V., 2016), determination of hypermobility of joints (by the Beighton method), bimanual vaginal examination, transvaginal ultrasound cervicometry. The diagnosis of CI was carried out based on data from a transvaginal ultrasound cervicometry (the length of the closed part of the cervix is less than 25 mm, the ratio of the length of the cervix to its diameter at the level of the internal pharynx is less than 1.2).

The study included typing of the examined patients by polymorphism *Arg25Pro* (915G>C; rs1800471 in places) of the *TGFß1* gene, and *A-8202G* (rs11697325) of the *MMP9* gene, as well as determination of the level of *TGFß1* and *MMP9* in blood serum. DNA from blood leukocytes was isolated using a commercial DNA-express-blood kit (NPF Litech, RF). Genotyping was carried out using appropriate commercial kits for detecting mutations (polymorphisms) in the human genome of the SNP-express kit (NPF Litech, RF) by allele-specific polymerase chain reaction with detection of results by horizontal electrophoresis in 3% agarose gel according to the standard protocol.

The levels of MMP9 and TGFß1 in blood serum was determined using commercial test systems "TGFß1", "MMP-9", (Elabscience Biotechnology Co., Ltd., China) by enzyme immunoassay (ELISA).

Data were evaluated for compliance with the normal distribution using the Shapiro-Wilk criteria. All the results of data obeyed the normal distribution law and are presented in the form of M ± SD, where M is the arithmetic mean, SD is the standard deviation. When analyzing the intergroup differences in quantitative characteristics between two independent groups, the Student's t-test was calculated. In order to compare the distribution of qualitative features in the studied groups, the Pearson criterion **χ**-squared (χ^2) with the Yates correction for continuity was used. Pearson's correlation coefficient rxy was used as an indicator of the closeness of the relationship between quantitative indicators having a normal distribution.

The distribution of genotypes across the polymorphisms studied was checked for compliance with the Hardy-Weinberg equilibrium using the Fischer test. Qualitative data are presented in the form of the number N and % (the number of patients carrying this allele and the percentage of their number in the group of subjects) or the decimal fraction of one (P). Intergroup differences in the frequency distribution of genotypes of polymorphic variants of the studied genes were determined using the Pearson criterion **χ**-square (χ^2). To analyze

the association of alleles and genotypes of the studied genes with the risk of developing a gestational complication, the odds ratio (OR) with 95% confidence intervals (CI) was calculated. In all types of statistical analysis, the differences were considered significant at $p < 0.05$.

Results and Discussion

The patients of both groups were comparable in age: the average age of patients in the main group was 28.9 ± 4.5 years, in the control group - 29.3 ± 5.4 years.

Among pregnant women of the main group were most common such phenotypic markers of dysplasia, as varicose veins (diagnosed in 52.7% of patients), mitral valve prolapse (44.4%), myopia (61.1%), splanchnoptosis (25%).

The leading place in the structure of complications of pregnancy in women of the main group was occupied by gestational complications associated with miscarriage: significantly more often in comparison with the control group, pregnant women with CI and CTD were diagnosed in the first trimester - threatening and incipient miscarriage (55.6% vs. 31.4%, OR = 1.8; 95% CI 1.01 - 3.12; p = 0.041), retrochorial hematoma (30.5% vs. 8.6%, OR = 3.4; 95% CI 1.05 - 11.35; p = 0.024), in the II and III trimesters, premature discharge of amniotic fluid was 2.4 times more common (OR = 2.43; 95% CI 1.23 - 4.77; p = 0.07).

In pregnant women of the main group, the average concentration of MMP9 in blood serum was 1.4 times higher than the value of this indicator in the control group (1.43 ± 0.34 ng/ml versus 1 ± 0.35 ng/ml, p = 0.000002), the level of serum concentration of TGFß1 in the main group also significantly exceeded the value of this indicator in the control group (32.2 ± 12.0 ng/ml versus 16.4 ± 5.7 ng/ml, $p < 0.0001$), which indicates enhanced collagenolysis in patients with CI on the background of connective tissue disorder.

We found an inverse moderate statistically significant correlation between the length of the cervix and the level of MMP9 in the blood serum in patients of the main group (r = -0.49, p = 0.0025) (Figure 1).

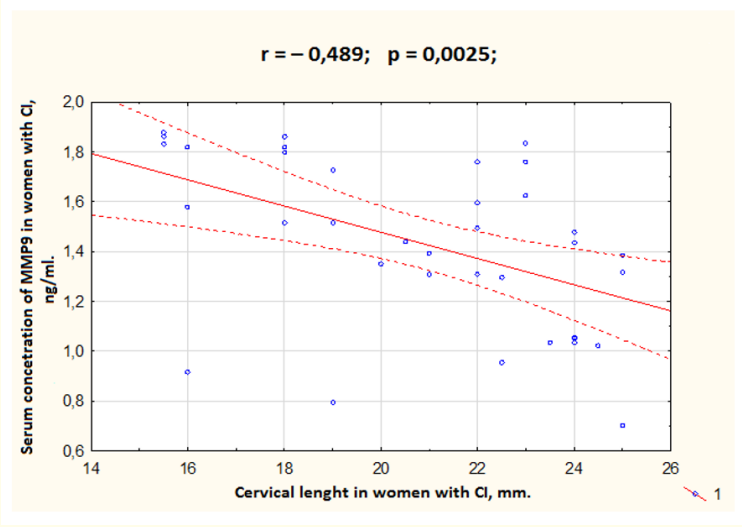

Figure 1: *Correlation between cervical length and serum concentration of MMP9 in patients with CI.*

The distribution of alleles and genotypes by polymorphic variants of the studied genes in both groups corresponded to the Hardy-Weinberg equilibrium. The obtained results of the prevalence of polymorphic alleles of the MMP9 and TGFß1 genes in women of the control group coincided with the corresponding data of the NCBI database (db SNP).

When analyzing the frequency distribution of genotypes by polymorphism A-8202G of the MMR9 gene, it was found, that the homozygous variant allele -8202G (G/G) genotype was statistically more common in the main group compared with the control group (OR = 4.00; 95% CI 1.23 - 12.98; p = 0.02). In general, the variant allele -8202G can be regarded as a "risk allele" for the development of CI in patients with CTD, since it was found that its presence in the genotype in both homo- and heterozygous states is associated with a higher incidence of this gestational complication (OR = 1.84; 95% CI 1.01 - 3.39; p = 0.04) (Table 1).

Genotype allele	Frequency, n (%)		χ^2	P, value	OR	95% CI
	With CI (n = 36)	Without CI (n = 35)				
MMP9, SNP rs11697325						
A/A	10 (27.7)	17 (48.6)	3.26	0.07	0.57	0.30-1.07
A/G	14 (38.9)	15 (42.8)	0.12	0.73	0.90	0.52-1.59
G/G	12 (33.4)	3 (8.6)	5.39	0.02*	4.00	1.23-12.98
TGFβ1, SNP rs1800471						
Arg/Arg	7 (19.4)	23 (66.7)	15.57	0.0001*	0.29	0.15-0.6
Arg/Pro	27 (75)	7 (20)	21.51	0.0001*	3.75	1.88-7.47
Pro/Pro	2 (5.6)	5 (14.3)	0.69	0.40	0.39	0.08-1.87

Table 1: *Genotype frequencies of MMP9 and TGFβ1 genes in cases and controls.*

** Fisher exact test.*

When analyzing the frequency distribution of genotypes according to the *Arg25Pro* polymorphism of the *TGFß1* gene, a statistically significantly higher frequency of occurrence was recorded in the main group compared to the control group of the heterozygous genotype (*Arg/Pro*) of this polymorphism (OR = 3.75; 95% CI 1.88 - 7.47; p < 0.0001). It is interesting to note, that the homozygous genotype *Arg25-*(*Arg/Arg*) was more common in the control group (OR = 0.29; 95% CI 0.15 - 0.6; p < 0.0001) (Table 2).

Serum Concentration, ng/ml	Genotype			P, value		
	I	II	III			
	A/A (n = 27)	A/G (n = 29)	G/G (n = 15)	P I-II	P I-III	PII-III
MMP-9	1.3 ± 0.2	1.4 ± 0.4	1.7 ± 0.2	0.88	0.0017*	0.007*
	Arg/Arg (n = 30)	Arg/Pro (n = 34)	Pro/Pro (n = 7)	P I-II	P I-III	PII-III
TGFβ1	21.2 ± 13.0	27.8 ± 12.1	23.2 ± 6.4	0.04*	0.82	0.21

Table 2: *Serum concentration of MMP9 and TGFβ1 depending on the genotype of the examined patients.*

** Fisher exact test.*

When studying the dependence of the MMP9 level in the blood serum of the examined patients on the genotype of the *A-8202G* polymorphism of the *MMP9* gene, it was found, that in patients with the presence of the "mutant" allele *-8202G* (*A/G* and *G/G* genotypes), the content of MMP9 was statistically significantly higher than in carriers of the *A/A* genotype, and it was 1.31 ± 0.43 ng/ml versus 1.08 ± 0.31 ng/ml (p = 0.021). The highest level of MMP9 was detected in pregnant women with the *G/G* genotype (Table 3).

Serum concentration, ng/ml	Genotype			t-Test; p		
	I	II	III			
	A/A (n = 27)	A/G (n = 29)	G/G (n = 15)	P_{I-II}	P_{I-III}	P_{II-III}
MMP-9	1.08 ± 0.31	1.31 ± 0.43	1.7 ± 0.23*	0.88	0.021*	0.007*

Table 3: *Serum concentration of MMP9 depending on the genotype of the examined patients.*
**Fisher exact test.*

When studying the effect of the *Arg25Pro* polymorphism genotypes of the *TGFß1* gene on the serum concentration of the protein encoded by it, was revealed the conjugation of the *Arg/Pro* genotype with a statistically significantly higher serum concentration of *TGFß1* in comparison with carriers of the homozygous genotype according to the *Arg25*-allele (Table 4).

Serum concentration, ng/ml	Genotype			t-Test; p		
	I	II	III			
	Arg/Arg (n = 30)	Arg/Pro (n = 34)	Pro/Pro (n = 7)	P_{I-II}	P_{I-III}	P_{II-III}
TGFβ1	21.16 ± 13.05	27.81 ± 12.04*	23.25 ± 6.37	0.039*	0.82	0.21

Table 4: *Serum concentration of TGFβ1 depending on the genotype of the examined patients.*
**Fisher exact test.*

Thus, the presence of "mutant" genotypes of the *MMP9* and *TGFß1* genes in patients with CTD is associated with a statistically significant increase in the serum concentration of the proteins encoded by them.

The obtained results allow us to consider these polymorphisms of the *TGFß1* and *MMP9* genes as genetic markers of the formation of CI in patients with CTD.

When analyzing the outcomes of pregnancy in the examined patients, it was revealed, that the frequency of premature birth in the main group was 36.11% versus 2.86% of cases in the control group (OR = 12.63; 95% CI 1.74 - 91.45; p = 0.0006). It is noteworthy that in the structure of preterm labor in patients with CI and CTD very early (up to 28 weeks) and early (from 28 weeks to 33 weeks 6 days) preterm labor prevailed - 7 (53.8%) cases, while the only preterm birth that took place in the control group were related to late (from 34 weeks to 36 weeks 6 days).

Conclusion

The association between polymorphism of the studied genes and cervical incompetency in pregnant women with undifferentiated connective tissue dysplasia was revealed.

In the main group were statistically significantly more often registered in comparison with patients of the control group the genotype *G/G* of polymorphism *A-8202G*, (rs11697325) *MMP9* gene and the genotype *Arg/Pro*of polymorphism *Arg25Pro* (rs1800471) *TGFß1* gene (OR = 4.00; 95% CI 1.23 - 12.98; p = 0.02; and OR = 3.75; 95% CI 1.88-7.47; p < 0.0001, respectively), which allows us to consider these genotypes as markers of a high probability of CI development in this cohort of patients.

In women of the control group was statistically significantly more common the *Arg/Arg* genotype of the polymorphism *Arg25Pro* (rs1800471) of the *TGFß1* gene (OR = 0.29; 95% CI 0.15 - 0.6; p < 0.0001), which can be regarded as a "protective" effect against the CI.

Thus, molecular typing of polymorphic variants *A-8202G* (rs11697325) of the *MMR9* gene and *Arg25Pro* (rs1800471) of the *TGFß1* gene will allow forming high-risk groups for the development of cervical insufficiency in patients with CTD at the pre-pregnancy stage and in the early stages of pregnancy, developing individual recommendations for the prevention and early diagnosis of this gestational complication, to increase the effectiveness of its treatment and, thus, improve obstetric and perinatal prognosis.

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Conflict of Interest

The author declares about absence conflict of interest.

Bibliography

1. Sidel'nikova VM and TG Sukhikh. "Nevynashivanie beremennosti". Moscow: MIA (2010): 536.
2. Vink J and H Feltovich. "Cervical etiology of spontaneous preterm birth". *Seminars in Fetal and Neonatal Medicine* 21.2 (2016): 106-112.
3. Warren JE., *et al*. "Collagen 1ά1 and Transforming Growth Factor-β Polymorphisms in Women with Cervical Insufficiency". *Obstetrics and Gynecology* 110.3 (2007): 619-624.
4. Petersen LK and N Uldbjerg. "Cervical collagen in non-pregnant women with previous cervical incompetence". *European Journal of Obstetrics and Gynecology and Reproductive Biology* 67.1 (1996): 41-45.
5. Schlembach D., *et al*. "Cross-linked collagen in the cervix of pregnant women with cervical insufficiency". *American Journal of Obstetrics and Gynecology* 189.6 (2003): S70.
6. Sundtoft I., *et al*. "Cervical collagen is reduced in non-pregnant women with a history of cervical insufficiency and a short cervix". *Acta Obstetricia et Gynecologica Scandinavica* 96.8 (2017): 984-990.
7. Gurbanova SR. "Vozmozhnosti optimizatsii akusherskoi taktiki vedeniya beremennosti i rodov u patsientok s istmiko-tservikal'noi nedostatochnost'yu i markerami nedifferentsirovannoi displazii soedinitel'noi tkani". *Vestnik RUDN Seriya Meditsina Akusherstvo i Ginekologiya* 6 (2009): 196-201.
8. Yoshida K., *et al*. "Cervical Collagen Network Remodeling in Normal Pregnancy and Disrupted Parturition in Antxr2 Deficient Mice". *Journal of Biomechanical Engineering* 136.2 (2014): 021017.
9. Frolov AL., *et al*. "Rol' markerov displazii soedinitel'noi tkani v razvitii istmiko-tservikal'noi nedostatochnosti pri beremennosti". *Mat' I Ditya V Kuzbasse* 3 (2014): 54-57.
10. Davidson L., *et al*. "Maternal race/ethnicity and cervical insufficiency". *American Journal of Obstetrics and Gynecology* 214.1 (2016): S441.
11. Nicholls-Dempsey L., *et al*. "Pregnancy outcomes in women with ehlers-danlos syndrome". *American Journal of Obstetrics and Gynecology* 220.1 (2019): S381-S382.

12. Natsional'nye klinicheskie rekomendatsii Rossiiskogo nauchnogo meditsinskogo obshchestva terapevtov po diagnostike, lecheniyu, reabilitatsii patsientov s displaziyami soedinitel'noi tkani. Materialy XII Natsional'nogo kongressa terapevtov Moscow (2017): 7-25.

13. Maslennikov AV., *et al*. "Techenie beremennosti u zhenshchin s nedifferentsirovannoi displaziei soedinitel'noi tkani". *Meditsinskii Vestnik Bashkortostana* 9.3 (2014): 55-58.

14. Athayde N., *et al*. "A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes". *American Journal of Obstetrics and Gynecology* 179.5 (1998): 1248-1253.

15. Botsis D., *et al*. "The value of cervical length and plasma proMMP-9 levels for the prediction of preterm delivery in pregnant women presenting with threatened preterm labor". *European Journal of Obstetrics and Gynecology and Reproductive Biology* 128.1-2 (2006): 108-112.

16. Rudoi AS. "TGF-beta-zavisimyi patogenez sindroma Marfana i rodstvennykh nasledstvennykh narushenii soedinitel'noi tkani". *Arterial'naya Gipertenziya* 15.2 (2009): 157-168.

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