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BRIEF REPORT

Analysis of molecular genetic markers of connective tissue dysplasia

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ABSTRACT

Introduction. Connective tissue dysplasia (CTD) is a hereditary, multifactorial condition characterized by impaired development of connective tissue during the embryonic and postnatal periods. This impairment results from genetically determined defects in the formation, maturation, and metabolism of cells and the extracellular matrix. The aim of this study was to investigate the associations of three polymorphic variants of the *ADAMTS5* gene with CTD in general, and with specific phenotypic features of CTD.

Materials and Methods. A cross-sectional study was conducted. The study included 181 participants (35 males, 19.3%, 146 females, 80.7%) with a mean age of 21.9 with a standard deviation of 2.9 years. At the first stage, all participants underwent a clinical examination, and signs of CTD were assessed using the

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Kadurina T.I. score, as modified by Tyurin A.V. The subsequent stage involved a molecular genetic analysis. Statistical data processing was performed using Excel 2024 and GraphPad Prism 8 software packages.
Results. The clinical examination, utilizing quantitative scoring methods, revealed signs of CTD in 130 subjects (71.8%). A comparative analysis of the allele and genotype frequency distributions for the *ADAMTS5* gene loci (rs226794, rs9978597, and rs2830585) revealed the following significant associations: the A allele and AA genotype of rs226794 with the presence of internal organ hernias (p=0.015 and p=0.007, respectively); the T allele and TT genotype of rs9978597 with CTD (p=0.003 and p=0.004, respectively); and the T allele and TT genotype with skin hyperelasticity (p=0.03 and p=0.03, respectively) and hypotension (p=0.015 and p=0.02, respectively).
Conclusion. Thus, the polymorphic variant rs226794 of the *ADAMTS5* gene is a risk marker for the development of internal organ hernias, while rs9978597 is a risk marker for CTD, skin hyperelasticity, and hypotension.

Key Words: *ADAMTS5*; extracellular matrix; biomarker; diagnosis; preventive medicine

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Introduction

Connective tissue dysplasia (CTD) is a hereditary, multifactorial disorder characterized by impaired development of connective tissue during embryonic and postnatal periods. This impairment stems from genetically determined defects in the formation, maturation, and metabolism of both cells and the extracellular matrix (ECM) [1, 2]. The genetic architecture of CTD remains a subject of ongoing research, as current knowledge is primarily based on phenotypic manifestations, with no unified molecular genetic diagnostic system established. Furthermore, given the ubiquitous presence of connective tissue in the human body, CTD is recognized as a predisposing factor for a wide range of associated pathologies [3, 4]. These include disorders of the musculoskeletal system, such as early-onset osteoarthritis (OA) and osteoporosis [5, 6], as well as conditions affecting the cardiovascular and gastrointestinal systems.

It is known that not only abnormalities in the structure of connective tissue components but also cellular apoptosis, inflammation, ECM degradation, and oxidative stress play a crucial role in the development and progression of CTD. The activity of most of these factors is regulated by enzymes involved in connective tissue degeneration, primarily matrix metalloproteinases. For instance, *ADAMTS5* belongs to the *ADAMTS* (A Disintegrin and Metalloproteinase with Thrombospondin Motifs) family of metalloproteinases, which are known for their role in ECM remodeling and proteolytic processes [7]. Aggrecanase-2, the enzyme encoded by the *ADAMTS5* gene, promotes ECM degradation, inflammation, and apoptosis in chondrocytes [8, 9] and may potentially contribute to the pathogenesis of CTD and its associated conditions. However, the available data remain fragmentary and contradictory. Therefore, investigating polymorphic variants of the *ADAMTS5* gene in the context of CTD remains a relevant endeavor, as it may uncover common metabolic pathways underlying CTD and its associated pathologies.

The aim of this study was to investigate the associations of three polymorphic variants of the *ADAMTS5* gene – rs226794, rs9978597, and rs2830585 – CTD in general, as well as with its specific phenotypic features.

Materials and Methods

Study design

A cross-sectional study was conducted. The study included 181 participants (35 males, 19.3%, 146 females, 80.7%) with a mean age of 21.9 with a standard deviation of 2.9 years. The study was performed in accordance with the Declaration of Helsinki (2013) and was approved by the Local Ethics Committee of the Bashkir State Medical University (Protocol № 11 15.11.2023). All participants received a detailed explanation of the study procedures in comprehensible language, and voluntary informed written consent was obtained from each individual.

The exclusion criteria were as follows: monogenic hereditary connective tissue disorders (Marfan syndrome, Ehlers-Danlos syndrome, osteogenesis imperfecta), autoimmune and autoinflammatory connective tissue diseases, decompensation of chronic conditions, acute infectious diseases, pregnancy, lactation, and refusal to participate.

At the first stage, all participants underwent a clinical examination. The presence of CTD signs was assessed using a quantitative scoring system based on the scale developed by Kadurina T.I. and modified by Tyurin A.V. [10]. This method assigns a score (0, 1, or 2) to each CTD feature according to its sensitivity and specificity, allowing for the evaluation of both the presence of individual CTD signs and the total cumulative score.

The subsequent stage involved molecular genetic analysis. A biomaterial sample of 10 ml of venous blood was collected from each participant. DNA extraction was performed using the standard phenol-chloroform method. The analysis of *ADAMTS5* gene polymorphisms was conducted by Real-time PCR. Genomic DNA was isolated from whole venous blood using phenol-chloroform extraction. DNA concentration was measured using an Epoch-1 spectrophotometer (BioTek, USA) and a Qubit fluorometer (Thermo Fisher Scientific, USA). To determine allele and genotype distributions for the investigated loci, real-time PCR was performed employing TaqMan and KASP technologies on a CFX96 Thermal Cycler (BioRad) and a QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific).

Statistical analysis

Hardy-Weinberg equilibrium was assessed using the HaploView 4.2 software package. Statistical data processing was carried out with Excel 2024 and GraphPad Prism 8 software. Quantitative traits were analyzed using the Chi-squared (χ^2) test with Yates› correction for 2x2 contingency tables. The strength of associations was estimated using odds ratios (OR) with 95% CI (confidence intervals) at a significance level of $p < 0.05$, and correction for multiple comparisons was performed using the Benjamini-Hochberg procedure.

Results

Clinical examination using quantitative scoring methods revealed signs of CTD in 130 participants (71.8%). The most frequently observed signs were

joint crepitus and body mass index (BMI) <18 kg/m^2, while the least common manifestations were chest wall deformities and internal organ hernias.

The characteristics of the investigated loci (rs226794, rs9978597, rs2830585) are presented in Table 1.

Table 1. Characteristics of the investigated *ADAMTS5* gene loci and analysis of conformity to Hardy-Weinberg equilibrium

Locus	Chromosomal position	Variant	Functional significance	Hpred	Hobs	HWpval	MAF	Alleles
rs226794	21:26930036 (GRCh38)	c.2075T>A	Missense variant	0.340	0.301	0.268	0.217	G:A
rs9978597	21:26921824 (GRCh38)	c.*2229A>C	3'- region, splicing site binding of miRNA	0.256	0.199	1.0	0.151	T:G
rs2830585	21:26932893 (GRCh38)	c.1841G>A	Missense variant	0.219	0.206	0.683	0.125	C:T

Note: p > 0.05 indicates no significant deviation from equilibrium.
GRCh38 – Genome Reference Consortium Human Genome build 38 Hobs – observed heterozygosity; Hpred – predicted heterozygosity; HWpval – p-value for Hardy-Weinberg equilibrium assessment; MAF – minor allele frequency.

Significant associations were identified for polymorphisms rs9978597 and rs226794 of the *ADAMTS5* gene. Specifically, the A allele and AA genotype of rs226794 were associated with internal organ hernias (p = 0.015 and p = 0.007, respectively). The T allele and TT genotype of rs9978597 showed associations with CTD (p = 0.003 and p = 0.004, respectively), as well as with skin hyperelasticity (p = 0.03 for both) and hypotension (p = 0.015 and p = 0.02, respectively). These significant associations are presented in Table 2.

Table 2. Significant associations between *ADAMTS5* gene polymorphisms and connective tissue dysplasia

Phenotypic Features	n	Allele Frequencies		Genotype Frequencies		
		***ADAMTS5* rs226794**				
		A	G	AA	AG	GG
Internal Organ Hernias+	6	7 (0.6)	5 (0.4)	1 (0.2)	5 (0.8)	0
Internal Organ Hernias-	132	57 (0.2)	207 (0.8)	8 (0.1)	41 (0.3)	83 (0.6)
p		0.015	-	0.007	-	-
OR (95% CI)		5.1 (1.6–16.6)	-	3.1 (0.3–29.0)	-	-
		***ADAMTS5* rs9978597**				
		T	G	TT	TG	GG
CTD+	109	194 (0.9)	24 (0.1)	89 (0.8)	16 (0.1)	4 (0.1)
CTD-	34	49 (0.7)	19 (0.3)	18 (0.5)	13 (0.4)	3 (0.1)
p		0.003	-	0.004	-	-
OR (95% CI)		3.1 (1.6–6.2)	-	3.9 (1.7–9.1)	-	-
Skin Hyperelasticity+	61	112 (0.9)	10 (0.1)	53 (0.86)	6 (0.1)	2 (0.04)
Skin Hyperelasticity -	81	130 (0.8)	32 (0.2)	54 (0.6)	22 (0.3)	5 (0.1)
p		0.03	-	0.03	-	-
OR (95% CI)		2.8 (1.3–5.9)	-	3.3 (1.4–8.0)	-	-
Hypotension +	64	118 (0.9)	10 (0.1)	56 (0.9)	6 (0.09)	2 (0.01)
Hypotension-	78	124 (0.8)	32 (0.2)	51 (0.6)	22 (0.3)	5 (0.1)
p		0.015	-	0.02	-	-
OR (95% CI)		3.1 (1.46.5)	-	3.7 (1.5–8.9)	-	-

Note: The table presents genotyping results corresponding to high quality, data from low-quality samples were excluded from the analysis, p-value after Benjamin-Hochberg correction. The data are presented as counts with frequency. No significant association between the ADAMTS5 rs2830585 polymorphism and connective tissue dysplasia was found.
CTD+ – presence of connective tissue dysplasia; hypotension+ – presence of hypotension; OR – odds ratios; skin hyperelasticity+ – presence of skin hyperelasticity; 95% CI – 95% confidence interval.

The highest number of associations was identified with the alleles and genotypes of the rs9978597 locus. In the CTD group, the T allele and TT genotype were predominant (p= 0.003, OR 3.1, 95% CI 1.6–6.2 and p= 0.004, OR 3.9, 95% CI 1.7–9.1, respectively) compared to the control group. The T allele and TT genotype were also predominant in the group of patients with skin hyperelasticity, reaching statistical significance (p = 0.03, OR 2.8, 95% CI 1.3–5.9 and p= 0.03, OR 3.3, 95% CI 1.4–8.0). Furthermore, the T allele and TT genotype were associated with hypotension, maintaining significance after correction for multiple comparisons (p= 0.015, OR 3.1, 95% CI 1.4–6.5 and p= 0.02, OR 3.7, 95% CI 1.5–8.9).

The A allele and AA genotype of rs226794 were observed more frequently in the group of patients with internal organ hernias compared to the control group, maintaining statistical significance after correction for multiple comparisons (p = 0.015, OR 5.1, 95% CI 1.6–16.6 and p = 0.007, OR 3.1, 95% CI 0.3–29.0, respectively). The CI for the risk estimate in carriers of the AA genotype was exceedingly wide (0.3–29.0). This interval crosses the null value of 1.0, indicating a lack of statistical significance for the association and precluding a definitive conclusion regarding either an increased or decreased risk. The primary reason for this imprecision in the estimate is the small subgroup size (n=6), which limited the statistical power of the analysis. Consequently, the findings pertaining to the AA genotype should be interpreted with utmost caution, and further studies with larger sample sizes are required to confirm or refute this association. These findings suggest that the *ADAMTS5* rs226794 polymorphism may serve as a risk marker for internal organ hernias. No significant associations were found for rs2830585 with either specific phenotypic features of CTD or with CTD in general.

Discussion

The authors identified associations between the T allele and TT genotype of the *ADAMTS5* gene polymorphism rs9978597 with CTD in general, as well as with specific phenotypic features of CTD, including skin hyperelasticity and hypotension. However, data on the potential contribution of this polymorphic variant to the development of CTD remain limited [11], and results from genome-wide association studies are currently unavailable. The *ADAMTS5* rs9978597 polymorphism was investigated by Perera et al. as a marker of severity for intervertebral disc herniations, but no significant associations were reported [12].

We identified associations between the A allele and AA genotype of the *ADAMTS5* gene locus rs226794 and the presence of internal organ hernias, but not with CTD in general. This locus has been actively studied as a marker for knee OA; however, statistical significance was not reached in either European [13] or Asian cohorts [14–16]. A 2018 meta-analysis summarizing data from 8 studies (10 cohorts) demonstrated no significant association between the *ADAMTS5* rs226794 polymorphism and the risk of degenerative musculoskeletal pathology overall [17], which is consistent with our findings.

According to a study by El Khoury L. et al. conducted on two independent Caucasian populations – a South African cohort comprising 115 patients and an Australian cohort comprising 60 patients – no statistically significant associations were found between alleles and genotypes of the rs226794 polymorphic locus and Achilles tendon pathology [18]. In contrast to these findings, a study by Perera R.S. et al. focusing on lumbar intervertebral disc degeneration (n=368) reported that the A allele of rs226794 was associated

with increased severity of the degenerative process [19], which aligns with our data.

In the present study, no significant associations were found for the *ADAMTS5* rs2830585 polymorphism with either phenotypic features of CTD or with CTD overall. Published data on the association between the rs2830585 locus and OA risk is contradictory and appear to depend on population-specific factors.

A meta-analysis by Huo J.Z. reported a trend towards an association of the A allele of rs2830585 with an increased risk of degenerative musculoskeletal diseases in the Asian population, although it did not reach statistical significance [17]. The study by Gu J. demonstrated that the T allele and TT genotype were associated with a reduced risk of OA overall [14]. However, Zhou X. et al. described an opposite association: the TT genotype was associated with a two-fold increased risk of OA compared to the CC genotype, while the T allele increased the risk by 39% compared to the C allele [15].

In contrast to the results obtained in Asian populations, a study by Canbek U. on a limited sample (95 OA patients and 80 controls) found no significant association between the rs2830585 polymorphism and OA [20].

Conclusion

Thus, the *ADAMTS5* rs226794 polymorphism represents a risk marker for the development of internal organ hernias, while rs9978597 serves as a risk marker for CTD, skin hyperelasticity, and hypotension.

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