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A VARIANT OF *TP53* GENE (RS 1625895, 13494G>A) IS ASSOCIATED WITH NEOPLASM LOCALIZATION IN PATIENTS WITH UTERINE LEIOMYOMA

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***Background*: Uterine leiomyoma (UL) is the most common benign neoplasm of the uterus. It is still unknown surely what exactly initiates transformation of the uterine myometrial cells into UL. *Aim*: To study the effect of the *TP53* gene variants on the risk of development and clinical features of UL. *Materials and Methods*: Case-control study was performed using molecular genetic analyses of variants rs1042522 (119 G>C) and rs1625895 (13494G>A) of *TP53* gene in patients with UL and comparison group of healthy women. *Results*: Investigated *TP53* gene variants were not associated with the risk of UL development. The patients with the 13494GG genotype (rs1625895) had significantly more often subserous UL ($p < 0.05$). In patients with heterozygous variant of *TP53* — 13494GA genotype (rs1625895) intramural UL was observed ($p < 0.05$). *Conclusions*: The rs1625895 (13494G>A) variant of *TP53* gene was associated with UL localization. The identified dependence of the UL localization on the *TP53* gene variant could be useful for personalized approach to treatment.**

***Key Words*: uterine leiomyoma, risk of development, *TP53* gene variants, neoplasm localization.**

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Uterine leiomyoma (UL) is the most common benign neoplasm of the uterus. According to various data, UL is highly prevalent in women all over the world [1]. Due to the high prevalence and concomitant symptoms that can significantly affect the quality of life (e.g., uterine bleeding, lower abdominal pain, impaired fertility), this disease is a significant problem for the health care system.

It is known that the development of UL is a complex, multi-stage and multifactorial process. Growth factors, cytokines, steroid hormones of the ovaries play a key role in its formation and development [2–4]. However, it is still unknown what exactly initiates the transformation of the uterine myometrial cells into UL. The pathophysiology and trigger mechanisms that contribute to the development of UL are not fully understood. In particular, UL origin could be associated with the mechanisms involved in cell cycle regulation and DNA repair.

Many studies focused on the associations between gene variants encoding regulatory proteins of the cell cycle and the risk of development of various tumors. Among the available data, a large number of research are devoted to the study of *TP53* gene variants, which encodes the p53 protein — a multifunctional transcription factor activated in response to stress factors: DNA damage, oncogene activation, hypoxia, etc. [5–7]. It was shown that high expression of p53 is found in 13% cases [8]. To date, more than 400 confirmed variants in the *TP53* gene have been identified [9]. Among them, the rs1042522 (G119C or Arg72Pro) and rs1625895 (G13494A) variants are the most significant and common. We have found few reports on a relationship between *TP53* variants and the risk of UL development, which have yielded contradictory results. Therefore, the aim of our research was to investigate the effect of the *TP53* gene variants on the risk of development and clinical features of UL.

MATERIALS AND METHODS

The study enrolled 63 patients with UL, the diagnosis was made in accordance with the standards of the Ministry of Public Health of Ukraine. The mean age of patients with UL was 38.4 ± 6.9 years, BMI — 25.3 ± 4.2. The duration of the disease in the examined patients with UL, who were under constant follow-up at the Department of Rehabilitation of Reproductive Function of Women at the Research Institute of Pediatrics, Obstetrics and Gynecology since 2015, ranged from 2 months to 15 years, and averaged 3.8 ± 0.6 years.

During the observation period, patients underwent a complete clinical and laboratory examination according to the WHO recommendations, which included a study of the nature of complaints, medical history, reproductive history, conducted therapy. Gynecological examinations were performed according to general schemes. The structure and topography of myomatous nodes, the features of their vascularization in patients were studied by the ultrasound examination

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Abbreviation used: UL – uterine leiomyoma.

on the MyLab Seven device with a transabdominal 4–8 Hz and a transvaginal sensor at a scanning frequency of 4–9 Hz. We used ICD 10, 1990 and FIGO, 2011 classifications.

The control group consisted of 41 apparently healthy women of the appropriate age (mean age — 38.5 ± 11.0 years, BMI — 24.6 ± 4.5), who were not diagnosed with the disease during a standard examination.

Informed consent was obtained from each participant included in the study before the collection of blood. The study was approved by the Ethics Committee of State Institution "Institute of Pediatrics, Obstetrics and Gynecology of NAMS of Ukraine".

The genomic DNA for molecular genetic studies was isolated from peripheral blood using a commercial "Quick-DNA Miniprep Plus Kit" (Zymo Research, USA) according to the manufacturer's protocol. The rs1042522 and rs1625895 variants of the *TP53* gene were determined using the PCR-RFLP method according to the previously published protocols [10, 11]. The studied gene regions were amplified using a commercial kit "DreamTaq Green PCR Master Mix" (Thermo Scientific, USA) and specific oligonucleotide primers (Metabion, Germany). The amplification products of DNA fragments of the *TP53* gene were subjected to hydrolytic cleavage using appropriate restriction endonucleases (Thermo Scientific, USA). Information on the sequence of primers and restriction endonucleases is presented in Table 1.

The digested products were separated using agarose gel electrophoresis and visualized on a UV transilluminator (Fig. 1, 2).

Statistical data processing was conducted using the Microsoft Excel Pro Plus 2016 and SPSS v.27 software. Genotype and allele frequencies in the study and control groups were compared using the χ^2 test. The studied parameters were checked for the normality of distribution by Kolmogorov — Smirnov test. In the case of a normal distribution, the significance of differences between the indicators was determined using Student's *t*-test, and in a distribution that differed from the normal one, the Mann-Whitney U-test was used. Differences were considered significant for all types of analysis at $p < 0.05$.

RESULTS

The distribution of the *TP53* gene variants in patients with UL and in the control group was analyzed (Table 2). No significant differences were found, indicating that the studied gene variants were not associated with a risk of developing the disease.

Another aspect of our study was to examine the possible influence of the studied variants of the *TP53* gene on the course of the disease. To do this, we analyzed the clinical parameters in the group of patients with UL depending on the genotypes (Table 3).

In the presence of the 119CC genotype, patients tended to develop the disease at a younger age as compared to the 119GG genotype, but the size of the dominant node was smaller. Patients with the 119GG genotype, on the other hand, developed the disease at an older age, but the size of the dominant node was larger. These features indicate that the potential multidirectional influence of the studied gene variants on the manifestation of the disease and its course is obvious.

Patients with subserous UL (FIGO stage VI–VII) significantly more frequently ($\chi^2 = 5.12$, $p = 0.02$) had the 13494GG genotype as compared to patients with intramural UL (FIGO stage III–V). In con-

Table 1. Summary of PCR-RFLP analysis

Variant	Primer sequence (5′ to 3′)	Anneal T, °C	Restriction enzyme	Size of amplicon and restriction fragments (bp)
TP53 rs1042522	CTGGTAAGGACAAGGGTTGG ACTGACCGTGCAAGTCACAG	60	BstUI	Amplicon: 397 119G: 166, 231 119C: 397
TP53 rs1625895	TGGCCATCTACAAGCAGTCA TTGCACATCTCATGGGGTTA	57	MspI	Amplicon: 404 13494G: 69, 335 13494A: 404

Note: fragments up to 80 bp in size in agarose gel are not clearly visualized.

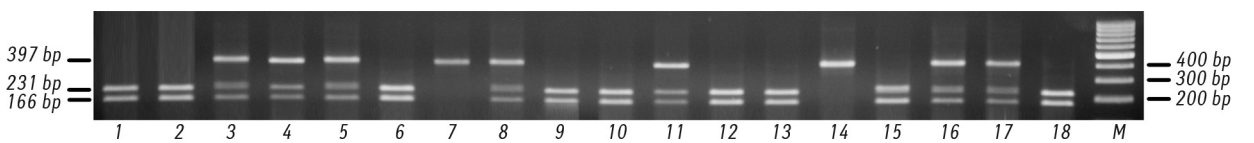

Fig. 1. Electrophoregram of restriction fragments for the rs1042522 variant of the *TP53* gene: 1, 2, 6, 9, 10, 12, 13, 15, 18 — 199GG genotype; 3–5, 8, 11, 16, 17 — 199GC genotype; 7, 14 — 199CC genotype; M — DNA marker

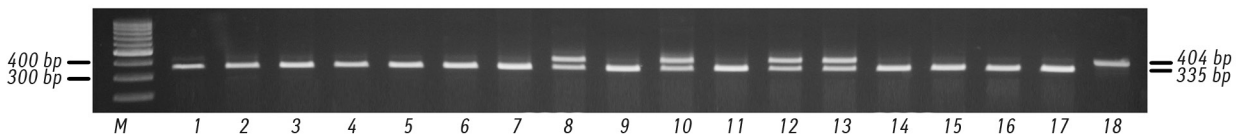

Fig. 2. Electrophoregram of restriction fragments for the rs1625895 variant of the *TP53* gene: 1–7, 9, 10, 11, 14–17 — 13494GG genotype; 8, 10, 12, 13 — 13494GA genotype; 18 — 13494AA genotype; M — DNA marker

Table 2. Comparison of the *TP53* gene variants distribution frequency in UL and control groups

Gene variants	Genotype, allele	UL group (n = 63)	Control group (n = 41)
TP53 rs1042522	119GG	30 (47.6%)	24 (58.5%)
	119GC	28 (44.4%)	12 (29.3%)
	119CC	5 (7.9%)	5 (12.2%)
	119G	0.70	0.73
	119C	0.30	0.27
TP53 rs1625895	13494GG	47 (74.6%)	32 (78.0%)
	13494GA	15 (23.8%)	6 (14.6%)
	13494AA	1 (1.6%)	3 (7.3%)
	13494G	0.87	0.85
	13494A	0.13	0.15

Note: $p > 0.05$ for all comparisons.

trast, a significantly increased frequency of the heterozygous 13494GA genotype ($\chi^2 = 4.29$, $p = 0.04$) was observed in patients with the intramural localization of the disease as compared to subserous localization.

For the rest of the studied clinical characteristics listed in Table 3, no significant differences were found.

DISCUSSION

Clinical application of the up-to-date techniques in genetic analysis helps to deepen our knowledge about the development and progression of various neoplasms. In this regard, SNP analysis is a powerful approach to extensive genomic screening of candidate genes that may be involved in the development of multifactorial diseases. p53 protein is involved in the regulation of cell cycle, cell growth and apoptosis. One of the most studied variants of the *TP53* gene is the G119C gene variant, which causes the proline to arginine substitution at codon 72 (CCC to CGC). As a result, two variants of p53, which have different biochemical properties, are formed. The genotypes of variant rs1042522 of *TP53* gene are significantly associated with different levels of p53 expression in cells [12]. According to the literature, the 119C variant is crucial for the specific activation of p53-dependent DNA repair genes in different cells, which leads to higher efficiency of DNA repair *in vitro* [13]. Perhaps that is why our patients with the 119CC genotype had a smaller size of the dominant node. In contrast, p53 protein formed by 119G variant is more effective inducer of apoptosis [14] and therefore the development of the disease in the examined patients occurred later. In addition, the rs1625895 variant is a common and possible biomarker, which is localized in the 6th intron of *TP53* gene and affects the function and expression of p53 [15]. The results of our research showed that this variant of the gene was associated with the localization of UL.

Analysis of the literature to study the possible impact of the rs1042522 and rs1625895 variants of the *TP53* gene on the risk of developing UL demonstrated quite contradictory results. In most studies, no relationship was found between the rs1042522 variant of the *TP53* gene and the risk of developing UL [16–18]. These data are completely consistent with our results. In contrast, studies by other authors have shown an association between the rs1042522 variant of *TP53* and the risk of developing UL [19–21]. This discrepancy in the results can be explained by the population difference in the frequencies of the rs1042522 variant of the *TR53* gene, or by the influence of other genetic or environmental factors. We did not find any data on the study of the role of the rs1625895 variant of the *TP53* gene in the development of UL, therefore, the detected features as to the distribution of this variant of the gene with different frequency at different localization of the tumor process are important for further clinical consideration.

To sum up, our study did not find an association of the rs1042522 and rs1625895 variants of the *TP53* gene with the risk of developing UL. The rs1625895 variant of the *TP53* gene was associated with UL localization. If patients had the 13494GG genotype, subserous UL was detected significantly more often, and in the heterozygous variant of the 13494GA genotype, intramural UL was observed. The identified association of UL localization on the *TP53* gene variant is an important prerequisite for a personalized approach to treatment.

Table 3. Comparison of genotypes with clinical characteristics of UL patients

	TP53 rs1042522			*TP53* rs1625895		
	119GG	119GC	119CC	13494GG	13494GA	13494AA
Age (y)	39.2 ± 7.8	37.8 ± 6.1	34.8 ± 0.8	38.3 ± 7.2	38.2 ± 5.8	36.0
Type of treatment						
Conservative	10 (41.7%)	11 (45.8%)	3 (12.5%)	19 (79.2%)	41 (16.7%)	1 (4.2%)
Surgical	19 (52.8%)	16 (44.4%)	1 (2.8%)	27 (75.0%)	9 (25.0%)	0 (0%)
Focality of myomatosis						
Unifocal	10 (50.0%)	9 (45.0%)	1 (5.0%)	16 (80.0%)	4 (20.0%)	0 (0%)
Multifocal	16 (44.4%)	17 (47.2%)	3 (8.3%)	26 (72.2%)	9 (25.0%)	1 (2.8%)
Size of the dominant node (mm)	55.1 ± 43.1	42.7 ± 26.0	29.0 ± 15.1	49.6 ± 37.7	41.9 ± 26.6	32.0
FIGO stage						
0–II	0 (0%)	2 (100.0)%	0 (0%)	1 (50.0%)	1 (50.0%)	0 (0%)
III–V	15 (44.1%)	15 (44.1%)	4 (11.8)	**22 (64.7%)***	**11 (32.4%)***	**1 (2.9%)***
VI–VII	12 (57.1%)	9 (42.9%)	0 (0%)	**20 (95.2%)***	**1 (4.8%)***	**0 (0%)***
Pathology of endometrium						
No	8 (30.8%)	16 (62.5%)	2 (7.7%)	17 (65.4%)	8 (30.8%)	1 (3.8%)
Yes	12 (60.0%)	7 (35.0%)	1 (5.0%)	17 (85.0%)	3 (15.0%)	0 (0%)
Complications of the principal diagnosis						
No	13 (52.0%)	12 (48.0%)	0 (0%)	21 (84.0%)	4 (16.0%)	0 (0%)
Yes	16 (45.7%)	15 (42.9%)	4 (11.4%)	25 (71.4%)	9 (25.7%)	1 (2.9%)

Note: *The difference in distribution of *TP53* rs1625895 genotypes is significant between patients with FIGO stage of UL – III–V and VI–VII, $p < 0.05$.

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ВАРІАНТ ГЕНА *TP53* (RS 1625895, 13494G>A) ПОВ'ЯЗАНИЙ ІЗ ЛОКАЛІЗАЦІЄЮ НОВОУТВОРЕННЯ У ХВОРИХ НА ЛЕЙОМІОМУ МАТКИ

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***Стан питання*:** Лейоміома матки (ЛМ) є найпоширенішим доброякісним новоутворенням матки. Досі достеменно невідомо, що саме ініціює перетворення клітин міометрія матки на ЛМ. ***Мета*:** Дослідити вплив варіантів гена *TP53* на ризик розвитку та клінічні особливості ЛМ. ***Матеріали та методи*:** Дослідження «випадок-контроль» проводили з використанням молекулярно-генетичного аналізу варіантів rs1042522 (119G>C) та rs1625895 (13494G>A) гена *TP53* у пацієнток з ЛМ та у жінок групи порівняння. ***Результати*:** Досліджені варіанти гена *TP53* не були асоційовані з ризиком розвитку ЛМ. У пацієнток з генотипом 13494GG (rs1625895) значно частіше відмічали субсерозну ЛМ ($p < 0.05$). А у пацієнток з гетерозиготним варіантом гена *TP53* генотип 13494GA (rs1625895) частіше виявляли інтрамуральну ЛМ ($p < 0.05$). ***Висновки*:** Варіант rs1625895 (13494G>A) гена *TP53* був асоційованим з локалізацією новоутворення. Виявлена залежність локалізації ЛМ від варіанту гена *TP53* є важливою передумовою персоналізованого підходу до лікування.

***Ключові слова*:** лейоміома матки, ризик розвитку, варіанти гена *TP53*, локалізація новоутворення.