

The Effects of *PON1* Gene Q192R Variant on the Development of Uterine Leiomyoma in Turkish Patients

RUKSET ATTAR¹, HANDE ATASOY², GÜLDAL İNAL-GÜLTEKİN³, ÖZLEM TİMİRCİ-KAHRAMAN³, SEDA GÜLEÇ-YILMAZ², ALTAY BURAK DALAN⁴, GAZİ YILDIRIM¹, ARZU ERGEN³ and TURGAY İSBİR⁵

¹Department of Gynecology, Yeditepe University Hospital, Istanbul, Turkey;

²Department of Molecular Medicine, Institute of Health Sciences, Yeditepe University, Istanbul, Turkey;

³Department of Molecular Medicine, Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey;

⁴Yeditepe University Hospital, Istanbul, Turkey;

⁵Department of Medical Biology, Faculty of Medicine, Yeditepe University, Istanbul, Turkey

Abstract. *Aim: This study aimed to analyze the relation between uterine leiomyoma (ULM) patients and p.Q192R polymorphism. Materials and Methods: ULM patients (n=76) and healthy women (n=103) were recruited from the Yeditepe University, Department of Gynecology and Obstetrics. The genotype and allele distribution of p.Q192R was analyzed by polymerase chain reaction and restriction fragment length polymorphism methods. Genotype and allele frequencies between study groups were calculated by the chi-square (χ^2) and Fischer's exact test. Results: The frequency of the B allele was lower in patients ($p<0.001$) and the AB genotype showed a decreased risk for ULM development ($p<0.001$). The variation was unrelated to ULM size and number. There was no significant difference between p.Q192R genotype frequencies and fibroid size and number. Conclusion: The heterogeneous AB genotype of *PON1* p.Q192R variation could be recognized as a low-risk parameter for the development of ULM in Turkish women.*

Uterine leiomyomas (ULM) are benign tumors of smooth muscle cells of the myometrium. They represent a common health problem and are estimated to be present in 20-30% of clinically reproductive women (1, 2). Ethnicity, nulliparity, obesity, diet, early menarche and age are reported among predisposing factors. Furthermore, oestrogen is proposed as the

primary promoter of ULM (3). However, a clear pathogenesis, including a genetic pathway, has not been yet described.

Paraoxonase (PON1) is a high-density-lipoprotein bound enzyme coded by the *PON1* gene located on 7q21-3 (Gene ID: 5444) and composed of 9 exons and 8 introns. PON1 is an arylesterase that mainly hydrolyzes highly toxic compounds, such as paroxon, and protects against low-density lipid oxidation (4, 5). The gene produces a 1,769-bp long mRNA (NM_000446.5) and a 355-aa protein (NP_000437.3) (4). One of the common single nucleotide polymorphism (SNP) widely studied of the *PON1* gene results in a missense mutation. The rs662 in exon 6 is caused by a change from A>G and leads to a change from glutamine (Q) to arginine (R) (p.Gln192Arg, p.Q192R, A192B) (6) and has been associated with low and high paraoxonase activity, respectively (7). *PON1* has been implicated in several cancer types and was predicted as a possible biomarker for early diagnosis, prognosis and pathogenesis (7-16)

The aim of the present study is to identify a possible correlation between ULM and *PON1* 192 polymorphism and, consequently, to evaluate whether the *PON1* 192 polymorphism could be suggested as a marker in the early diagnosis and possibly improve quality of life of affected women.

Materials and Methods

This study comprised 2 groups in which all women had pelvic imaging to detect the presence or absence of ULM. Patients with ULM (n=76) and control volunteers (n=103) were assessed with 3 perpendicular diameters for the measurement of each fibroid: length, width and depth. Patients were further grouped according to the tumor number, being either one or multiple; the latter including two up to 13 separate tumours. A second grouping was made by fibroid size, with the threshold selected at 5 cm. The presence of pelvic pain and abnormal bleeding was also noted; however, not all women showed these signs. This study was approved by the Yeditepe University's Ethical Committee.

Correspondence to: Professor Dr. Turgay İsbir, Chairman, Department of Medical Biology, Faculty of Medicine, Yeditepe University, Yeditepe Üniversitesi Yerleşimi, İnönü Cad. 26 Ağustos Yerleşkesi, 34755 Kayışdağı-Ataşehir, İstanbul, Turkey. Tel: +90 5332823726/+90 21657800001263, e-mail: turgay.isbir@yeditepe.edu.tr and tisbir@superonline.com

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DNA isolation and genotyping for *PON 192* polymorphism. Peripheral blood (10 ml) was collected into EDTA tubes from Istanbul University and Yeditepe Universities, Departments of Gynecology and Obstetrics. Genomic DNA was extracted from peripheral whole blood using the iPrep PureLink gDNA Blood Kit with the iPrep Purification Instrument (Invitrogen, Life Technologies, Grand Island, NY, USA). Consequently, DNA samples were quantified (μg) and qualified (260/280 and 260/230) with a Nanodrop. The DNA samples were stored at $+4^\circ\text{C}$ until genomic studies were conducted.

The *PON1* p.Q192R variant was amplified using sense 5'-TAT TGT TGC TGT GGG ACC TGA G-3' and antisense 5'-CAC GCT AAA CCC AAA TAC ATC TC-3' primer sets, containing the 192 polymorphic region of the human *PON1* gene. The PCR reaction mixture contained 100 ng of DNA template, 0.5 μM of each primer, 1.5 mM of MgCl_2 , 200 μM of dNTPs and 1U of Taq DNA polymerase (Bioron, Ludwigshafen, Germany). Following denaturing the DNA for 5 minutes at 94°C , the reaction mixture was subjected to 35 cycles of denaturation for 1 minute at 95°C , 1 minute annealing at 60°C and 1 minute of extension at 72°C (17). The 99-bp PCR product was digested with 8 U BspPI restriction endonuclease (Thermo Fisher Scientific, Waltham, MA USA) overnight at 55°C and the digested products were separated by electrophoresis on a 3% agarose gel and visualized using ethidium bromide. The B genotype contains a unique *BspI* restriction site, which results in 66-bp and 33-bp products, whereas the A genotype is not cut, thus allowing the 192 genotype to be determined.

Statistical analysis. Frequency and statistical analysis were performed with the SPSS 20.0. software (SPSS Inc, Chicago, IL, USA) Data were presented as mean \pm standard deviation (SD) or as proportions. A $p < 0.05$ was defined as statistically significant. Expected and observed frequencies of genotypes and alleles were compared with Chi-Square analysis and Fisher's exact tests. Nominal values were analysed with the Student's *t*-test.

Results

A total of 76 patients with ULM and 103 controls were recruited for this study. The mean age of the patients and healthy controls were 35.38 ± 6.98 and 37.40 ± 9.17 years, respectively. No significant difference was found between patients and controls in terms of median age ($p = 0.220$).

The genotype and allele frequencies of the *PON Q192R* gene polymorphism between patient and control groups are shown in Table I. The frequency of the B allele was found to be significantly lower in patients compared to controls ($\chi^2 = 17.065$, $p < 0.001$, odds ratio (OR) = 0.409, 95% confidence interval (CI) = 0.255-0.657). A significant decreased risk for developing ULM was found in the AB genotype compared with the other genotypes ($\chi^2 = 25.319$, $p < 0.001$, OR = 0.231, 95% CI = 0.116-0.459).

Distributions of *PON Q192R* genotypes in relation to clinical parameters of ULM patients were examined. There were no significant differences between *PON1 Q192R* polymorphism genotype frequencies and uterine fibroid range in size and number ($\chi^2 = 0.714$; $p = 0.700$ for fibroid size, $\chi^2 = 1.354$; $p = 0.508$ for fibroid number).

Discussion

ULM represents one of the uterine smooth muscle tumours that are histologically divided into 6 types, including ULM, mitotically active leiomyoma, cellular leiomyoma, atypical leiomyoma, uncertain malignant potential and leiomyosarcoma (18). There exists some evidence that rare uterine leiomyosarcomas, occurring only in 0.1-0.3% of women, may in fact arise from benign ULMs (18, 19) or other tumorous types indicating that similar chromosomal aberrations are seen in both pathologies (19). Furthermore, ULM has also been implicated with increased risk of endometrial cancer (20).

Their non-malignant nature led to insufficient research (3); however, studies mainly published from the US showed in fact its burden on the health care system, representing over US\$ 2 billion in 2006 (21) and almost US\$ 6 to 35 billion in 2012 (22). One of the reasons for this considerably high amount of expenditure is due to the race factor, for it is well-documented that the black race is more susceptible to develop ULM compared to the white population (23). Thirty to seventy percent of reproductively active women, as young as in their mid-twenties, are at risk of developing ULM. Estrogen exposure, early age at menarche, obesity, physical activity, age, diet, parity, menopause, hormonal replacements, race and genetic factors are the major etiology of ULM (3, 23, 24). Heritability studies conducted in several European populations showed that 26-69% of ULM is due to genetic factors (25).

Genes involved in the estrogen metabolism, such as *COMT*, *CYP1A1* and *CYP1B1*, have been investigated in the Han Chinese population; it was concluded that the first two genes had protective effects on the formation and growth of uterine fibroids in UML (26). Edwards and coworkers (25) conducted a genome-wide association study in the European-American population and found a strong association between ULM risk and *BETIL* and *TNRC6B* genes (25). Additionally, chromosomal structural aberrations have recently been reported in ULM patients, including deletions, breaks and fragilities in several chromosomes (27). Nevertheless, specific pathways and potential gene interactions are yet to be discovered.

The *PON1* gene has been investigated for several cancer types in different populations, including prostate (13), lung (14), non-Hodgkin's lymphoma (15) and multiple myeloma (16). The 192R mutant (BB) is reported to be a risk factor for bladder, lung, multiple myeloma and non-Hodgkin's lymphoma increasing the risk almost three times for the latter. The *PON1 Q192R* (rs662) polymorphism was investigated in lung cancer (9) and bladder cancer patients (7) in the Turkish population. The mutant B allele was similarly found to be significantly associated with invasive growth pattern, perineural invasion and death for bladder

Table I. The distribution of the *PON1* Q192R (rs662) genotypes and allelic frequency in the study groups.

<i>PON1</i> Q192R(rs662)	ULM (n=76)	%	Control (n=103)	%	Total (n)	p-Value
Genotype						
AA	60	78.9	50	48.5	110	*<0.001
AB	8	10.5	47	45.6	55	
BB	8	10.5	6	5.8	14	
Allele						
A allele	128	84.21	147	72.35	275	0.074
B allele	24	15.79	59	27.65	83	*<0.001

ULM, Uterine leiomyoma; n, number of individuals; values are reported as number of patients (percentage of the total group). *p-Values less than 0.05 denote statistical significance.

cancer patients (7). A possible explanation for the BB mutant genotype to increase cancer risk could be that the lower metabolism rate of *PON1* causes accumulation of lipid-soluble free radicals (5).

Moreover, *PON1* Q192R has been investigated for breast cancer in Egypt and Caucasians in the US. In contrast to the above mentioned cancer types, the 192R (B) allele showed no effect and decreased risk of breast cancer risk, respectively (11, 12). Our results are in accordance with these findings with a significantly low occurrence of the mutant B allele in ULM patients compared to healthy controls. Heterozygote AB patients showed a decreased risk in the development of ULM. Additionally, patients with ovarian cancer showed an increased risk for the wild-type allele (A) in a Turkish cohort (8). This distinctive p.Q192R genotype variance between female reproductive organ cancers and other cancer types might be a pattern yet to be clarified. However, this hypothesis requires a thorough investigation with higher number of volunteers, involving other uterine malignant diseases and comparative population studies.

Although being rarely associated with mortality, it is reported that ULM might have an impact on the quality of life (QOL). Urinary incontinence, excessive bleeding and pelvic pain are some of the genitourinary signs observed in patients with ULM, affecting QOL. The size of fibroids was found to be a significant factor affecting urinary incontinence, along with physical activity and psychological health in patients with fibroid size equal to or higher than 5 cm (2). Infertility and pregnancy complications are also among the clinical signs and require therapy (28). The numbers of fibroids are clinically evaluated as single or multiple fibroids. The size and number of fibroids are suggested to be explained by genetic factors (23), while *PON1* activity was proposed as a possible marker for metastasis or growth of some tumors (7). However, our results did not have a significant effect on ULM size and number. Our group recently investigated the *ACE* gene insertion/deletion (I/D) polymorphism in ULM patients. Despite the correlation between *ACE* activity and tumor growth (29), the importance of angiogenesis on the

formation of ULM (30-32) and the dense vascular capsule of fibroids (32), there was no significant association between *ACE* I/D polymorphism among the size and number of fibroids. However, Ateş and colleagues determined that *COMT* Val158Met polymorphism was relevant on the formation of large fibroids (>5cm) but the variation was not attributed to an increased risk of ULM development in a Turkish cohort (33).

In summary, the heterogenous AB genotype of *PON1* Q192R variation could be recognized as a low-risk parameter for the development of ULM in Turkish women. Further studies need to be designed in order to identify potential genes and pathways affecting the formation, size and number of ULM. One possible preparatory work to wetlab techniques would be to design a bioinformatic study for the identification of all possible related genes and proteins to ULM, although whole -genome or- exome sequencing studies would also identify related genes in different populations.

References

- 1 Turan MC and Büyükbayrak EE: Myomalarda tedavi seçenekleri. *TJOD Uzmanlık Sonrası Eğitim Dergisi* 8: 163-169, 2004.
- 2 Ekin M, Cengiz H, Öztürk E, Kaya C, Yasar L and Savan K: Genitourinary symptoms and their effects on quality of life in women with uterine myomas. *Int Urogynecol J* 25: 807-810, 2014.
- 3 Flake GP, Andersen J and Dixon D: Etiology and pathogenesis of uterine leiomyomas: a review. *Environ Health Perspect* 111(8): 1037-1054, 2003.
- 4 National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/gene/5444>. (Accessed: December 2014)
- 5 Wysocka A, Cybulski M, Berbec H, Wysocki A, Stazka J and Zapolski T: Prognostic value of paraoxonase 1 in patients undergoing coronary artery bypass grafting surgery. *Med Sci Monit* 20: 594-600, 2014.
- 6 Lurie G, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, Terada KY and Goodman MT: Genetic polymorphisms in the paraoxonase 1 gene and risk of ovarian epithelial carcinoma. *Cancer Epidemiol Biomarkers Prev* 17(8): 2070-2077, 2008.

- 7 Ozturk O, Kağnıcı OF, Ozturk T, Durak H, Tuzuner BM, Kısakesen HI, Cakalır C and Isbir T: 192R Allele of Paraoxanase 1 (PON1) Gene as a New Marker for Susceptibility to Bladder Cancer. *Anticancer Research* 29: 4041-4046, 2009.
- 8 Arpacı A, Görmüş U, Dalan B, Berkman S and Isbir T. Investigation of PON1 192 and PON1 55 Polymorphisms in Ovarian Cancer Patients in Turkish Population. *In Vivo* 23: 421-424, 2009.
- 9 Aksoy-Sagırlı P, Cakmakoğlu B, Isbir T, Kaytan-Sağlam E, Kızır A, Topuz E and Berkkan H: Paraoxanase-1 192/55 Polymorphisms and the Risk of Lung Cancer in a Turkish Population. *Anticancer Res* 31: 2225-2230, 2011.
- 10 Stevens VL, Rodriguez C, Pavluck AL, Thun MJ and Calle EE: Association of polymorphisms in the paraoxanase 1 gene with breast cancer incidence in the CPS-II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev* 15: 1226-1228, 2006.
- 11 Gallicchio L, McSorley MA, Newschaffer CJ, Huang HY, Thuita LW, Hoffman SC and Helzlsouer KJ: Body mass, polymorphisms in obesity-related genes, and the risk of developing breast cancer among women with benign breast disease. *Cancer Detect Prev* 31: 95-101, 2007.
- 12 Hussein YM, Gharib AF, Etewa RL and ElSawy WH: Association of L55M and Q192R polymorphisms in paraoxanase 1(PON1) gene with breast cancer risk and their clinical significance *Mol Cell Biochem* 351: 117-123, 2011.
- 13 Antognelli C, Mearini L, Talesa VN, Giannantoni A and Mearini E: Association of CYP17, GSTP1, and PON1 polymorphisms with the risk of prostate cancer. *Prostate* 63: 240-251, 2005.
- 14 Lee CH, Lee KY, Choe KH, Hong YC, Kim YD, Kang JW and Kim H: Effects of oxidative DNA damage induced by polycyclic aromatic hydrocarbons and genetic polymorphism of the paraoxanase-1 (PON1) gene on lung cancer. *JPrev Med Pub Health* 38: 345-350, 2005.
- 15 Kerridge I, Lincz L, Scorgie F, Hickey D, Granter N and Spencer A: Association between xenobiotic gene polymorphisms and non-Hodgkin's lymphoma risk. *Br JHaematol* 118: 477-481, 2002.
- 16 Lincz LF, Kerridge I, Scorgie FE, Bailey M, Enno A and Spencer A: Xenobiotic gene polymorphisms and susceptibility to multiple myeloma. *Haematologica* 89: 628-629, 2004.
- 17 Adkins S, Gan KN, Mody M and La Du BN: Molecular basis for the polymorphic forms of human serumparaoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am J Hum Genet* 52(3): 598-608, 1993.
- 18 Zhang Q, Ubago J, Li L, Guo H, Liu Y, Qiang W, Kim JJ, Kong B and Wei JJ: Molecular analyses of 6 different types of uterine smooth muscle tumors: Emphasis in atypical leiomyoma. *Cancer* 120(20): 3165-3177, 2014.
- 19 Verit FF and Yucel O: Endometriosis, leiomyoma and adenomyosis: the risk of gynecologic malignancy. *Asian Pac J Cancer Prev* 14(10): 5589-5597, 2013.
- 20 Rowlands IJ, Nagle CM, Spurdle AB and Webb PM: Gynecological conditions and the risk of endometrial cancer. *Gynecol Oncol* 123: 537-541, 2011.
- 21 Flynn M, Jamison M, Datta S and Myers E: Health care resource for uterine fibroid tumors in the United States Michael. *American Journal of Obstetrics and Gynecology* 195: 955-964, 2006.
- 22 Cardozo ER, Clark AD, Banks NK, Henne MB, Stegmann BJ and Segars JH: The estimated annual cost of uterine leiomyomata in the United States. *Am J Obstet Gynecol* 206(3): 211.e1-9, 2012.
- 23 Baird DD, Dunson DB, Hill MC, Cousins D and Schectman JM: High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol* 188: 100-107, 2000.
- 24 Catherino WH, Eltoukhi HM and Al-Hendy A: Racial and ethnic differences in the pathogenesis and clinical manifestations of uterine leiomyoma. *Semin Reprod Med* 31(5): 370-379, 2013.
- 25 Edwards TL, Michels KA, Hartmann KE and Velez Edwards DR: BET1L and TNRC6B associate with uterine fibroid risk among European Americans. *Hum Genet* 132(8): 943-953, 2013.
- 26 Shen Y, Ren ML, Xu J, Xu Q, Ding YQ, Wu ZC, Zhang HB, Huang XX and Cai YL: A multicenter case-control study on screening of single nucleotide polymorphisms in estrogen-metabolizing enzymes and susceptibility to uterine leiomyoma in han chinese. *Gynecol Obstet Invest* 77(4): 224-230, 2014.
- 27 Hakverdi S, Demirhan O, Tunc E, Inandiklioglu N, Uslu IN, Gungoren A, Erdem D and Hakverdi AU: Chromosome imbalances and alterations in the p53 gene in uterine myomas from the same family members: familial leiomyomatosis in Turkey. *Asian Pac J Cancer Prev* 14(2): 651-658, 2013.
- 28 Cha PC, Takahashi A, Hosono N, Low SK, Kamatani N, Kubo M and Nakamura Y: A genome-wide association study identifies three loci associated with susceptibility to uterine fibroids. *Nat Genet* 43(5): 447-450, 2011.
- 29 Fishchuk LE and Gorovenko NG. Genetic polymorphisms of the renin-angiotensin system in breast cancer patients. *Exp Oncol* 35(2): 101-104, 2013.
- 30 Risau W. Mechanisms of angiogenesis. *Nature* 386(6626): 671-674, 1997.
- 31 Di Lieto A, De Falco M, Pollio F, Mansueto G, Salvatore G, Somma P, Ciociola F, De Rosa G and Staibano S: Clinical response, vascular change, and angiogenesis in gonadotropin-releasing hormone analogue- treated women with uterine myomas. *J Soc Gynecol Investig* 12: 123-128, 2005. (abstract)
- 32 Tal R and Segars JH. The role of angiogenic factors in fibroid pathogenesis: potential implications for future therapy. *Hum Reprod Update* 20(2): 194-216, 2014.
- 33 Ateş O, Demirturk F, Toprak M and Sezer S: Polymorphism of catechol-o-methyltransferase and uterine leiomyoma. *Mol Cell Biochem* 375(1-2): 179-183, 2013.

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