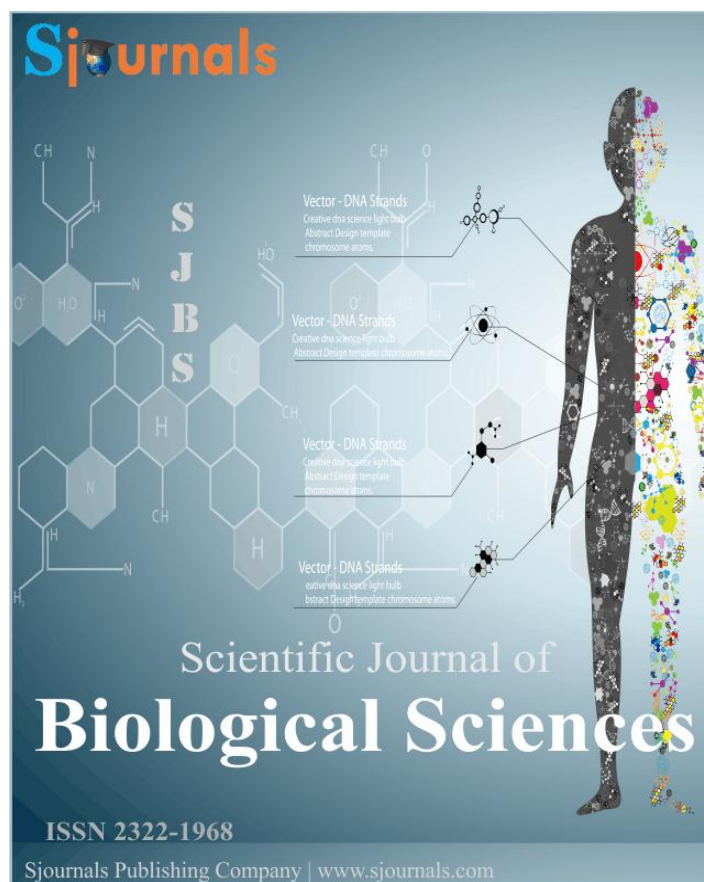


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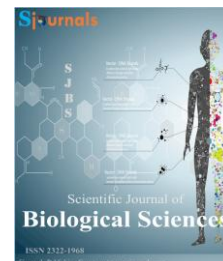
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**Original article**

**MED12 exon 2 mutations in uterine leiomyoma's in women patients of Tehran Province of Iran**

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ARTICLE INFO

*Article history,*

Received 13 December 2015

Accepted 12 January 2016

Available online 19 January 2016

iThenticate screening 16 December 2015

English editing 10 January 2016

Quality control 16 January 2016

*Keywords,*

Uterine Leiomyomas

Fibroid

MED12 Exon 2

Tumor

Mutation

ABSTRACT

Uterine leiomyomas or fibroids are benign smooth muscle tumors of myometrial origin; frequently found in reproductive age women. Uterine leiomyomas commonly cause significant symptoms such as anemia resulting from heavy irregular uterine bleeding, Pelvic discomfort, and bowel/bladder dysfunction from pressure. Fibroids have also been associated with infertility and recurrent abortion. Somatic mutations in MED12 exon 2 have recently been reported in uterine leiomyomas. The aim of our study was to see whether MED12 exon 2 mutations occur in uterine leiomyomas of women patients of Tehran Province of Iran. Mutations in exon 2 of the MED12 gene have been reported in 50% to 70% of uterine leiomyomas. To determine the frequency of MED12 mutations in various types of smooth muscle tumors as well as normal uterine myometrium adjacent to a leiomyoma, we selected a total of 23 cases for analysis of MED12 exon 2 mutations by polymerase chain reaction and Sanger sequencing. We found that 65% of classical uterine leiomyomas harbored mutations in exon 2 of the MED12 gene. Twelve of these mutations were located in codon 44 (c.130-132). In addition, one fibroids 0/43% displayed a missense mutation in codon 36 (c.107). We also observed two (0/86) exonic deletion type.

## 1. Introduction

Uterine leiomyomas, also called fibroids, are benign tumors that occur in 60% of women by the age of 45 years. That is a benign smooth muscle neoplasm that occurs with a cumulative incidence of approximately 77% based on pathological specimens (Cramer and Patel, 1990).

Approximately half of all leiomyomas are asymptomatic, while the rest cause pelvic pressure and pain, menorrhagia, anemia, premature labor and infertility. These symptoms are intensified by the common occurrence of multiple tumors within a single uterus (Guarnaccia and Rein, 2001). Although benign, leiomyoma morbidity can be high and management can be expensive. The estimated direct costs per year for medical and surgical treatment of leiomyoma were estimated at over \$3 billion in 2010 dollars (Flynn, et al., 2006). Similar to breast and ovarian cancers, uterine leiomyomata tend to grow under the influence of sex steroids such as estrogens and partly regress when the hormonal levels decrease (Di et al., 2008; Lethaby and Vollenhoven, 2005). This is frequently observed during pregnancy when the estrogen level rises and is followed by regression after delivery. Women are therefore routinely investigated for the occurrence of uterine leiomyomata by sonographic examination. However, with this assessment small-sized leiomyomata are extremely difficult to detect and are often overseen by the physician (Fauconnier et al., 2000). Current treatment options include hormonal suppression, embolization, and surgical management by myomectomy or hysterectomy. The pathogenesis of these tumors is incompletely understood, but experimental work has suggested involvement by particular molecular pathways. It is well documented that fibroids can be subdivided based on the existence of clonal chromosomal aberrations as e.g. deletions of the long arm of chromosome 7, trisomy 12, or chromosomal rearrangements targeting either of the two human HMGA gene loci (Velagaleti et al., 2010; Parker, 2007). Makinen *et al.*'s study is the first report of such frequent alterations identified in 70% of leiomyomas. All mutations are located in the intron 1 and exon 2 of MED12 (6.2% and 64.4% respectively) and are assumed to be activating mutations. The Mediator complex consisting of 26 subunits, seems to be implicated in transcription regulation and act as a bridge between DNA binding transcription factors and the RNA polymerase II transcription factors thus performing both general as well as gene-specific roles to activate or repress gene transcription (Taatjes, 2010; Conaway and Conaway, 2011). Med12 is part of one of the subunits participating in the formation of Mediator and, more specifically, its CDK8 submodule. Alterations of MED12 that has been assigned to Xq13.1, are known to cause the Opitz-Kaveggia and Lujan-Fryns syndrome (Risheg et al., 2007; Schwartz et al., 2007) both associated with X-linked mental retardation. In the present study, we sequenced a set of 23 uterine leiomyomas from Iranian woman patients. Sixty five leiomyomas (65%) harbored a mutation in MED12 exon 2.

## 2. Materials and methods

### 2.1. Tumor samples

A total of 23 formalin-fixed, paraffin-embedded (FFPE) tissue samples of smooth muscle tumors from 23 different women who were diagnosed with uterine leiomyoma's and underwent medically indicated abdominal hysterectomy were selected for DNA extraction and mutation analysis and were retrieved from the archives of the Department of Pathology at SHOHADA TAJRISH hospital (Tehran, Iran), from October 2009 to August 2012.

### 2.2. Tissue retrieval and DNA extraction

For all 23 cases, paraffin-embedded blocks were examined for adequate tissue volume, defined roughly as an area at least twice that of a 1 mm micro-punch. Only one block per case was used, and within that block, one contiguous area of tumor was selected for analysis. One-millimeter disposable sterile micro-punches were used to punch tissue cores from marked paraffin embedded blocks. Between 3 and 7 1-mm cores per block were obtained. Tissue cores were first deparaffinized and proteinase K-digested according to the manufacturer's instructions. DNA was extracted using a commercially available DNA extraction Rima Pure FFPE kit (Recover all Total Nucleic Acid Extraction Kit for formalin fixed, paraffin-embedded Tissues, Life Technologies, Grand Island, NY) that uses a

spin column–based extraction technique to maximize nucleic acid purity. Final DNA concentrations were determined using a Nano-drop 1000 spectrophotometer. Samples were included for PCR amplification and sequencing if the DNA concentration was greater than 6 ng/μL in the 50 μL DNA extraction volume

### 2.3. PCR and Sanger sequencing

At least 200 ng of DNA was PCR amplified using forward and reverse primers that targeted exon 2 of the MED12 gene resulting in a 291 bp PCR product. Primer sequences were as follows: 5'-GCCCTTACCTTGTCCTT-3' (forward) and 5'-TGTCCCTATAAGTCTTCCAACC-3' (reverse). The PCR reactions were carried out in a reaction volume of 25 μl containing genomic DNA 30–40 ng, 1 mol/l dNTP 2 μl, 25 mM MgCl<sub>2</sub> 1.5 μl, 10x PCR Buffer 2.5 μl, 10 pM primer 0.5 μl each, 1 U Taq polymerase (CinnaGen) 0.4 μl, and DDH<sub>2</sub>O. The reactions were carried out in the following thermocycler conditions: denaturation at 94C for 5 min, 36 cycles of 94C for 30 s, 63C for 30 s, and 72C for 30 s, and final elongation step at 72C for 10 min. PCR products were visualized by gel electrophoresis and staining with ethidium bromide in a digital camera system.

### 3. Results and discussion

A set of 23 uterine leiomyomas from *women patients of Tehran Province of Iran* was sequenced for MED12 exon 2 mutations to study the role of MED12 in tumorigenesis of fibroids. MED12 mutations were demonstrated in 15 of 23 leiomyomas (65%). twelve of these mutations were located in codon 44 (c.130-132). In addition, one fibroids (0/43%) displayed a missense mutation in codon 36. (c.107-), we also observed two (0/86) exonic deletion type. All three mutations are predicted to result in an in-frame transcript. The somatic nature of the mutations was verified in all cases where normal tissue DNA was available. (8) Eight patients did not have any mutations in MED12 exon 2. (Table 1)

Our results emphasize the major role of MED12 mutations in the genesis of these lesions and suggest that the mutations alone may be the key event in tumor development. Uterine leiomyomas provide an excellent example of tumors that are rather stable on nucleotide level and appear to arise through extremely specific missense mutations (Nilbert and Heim, 1990).

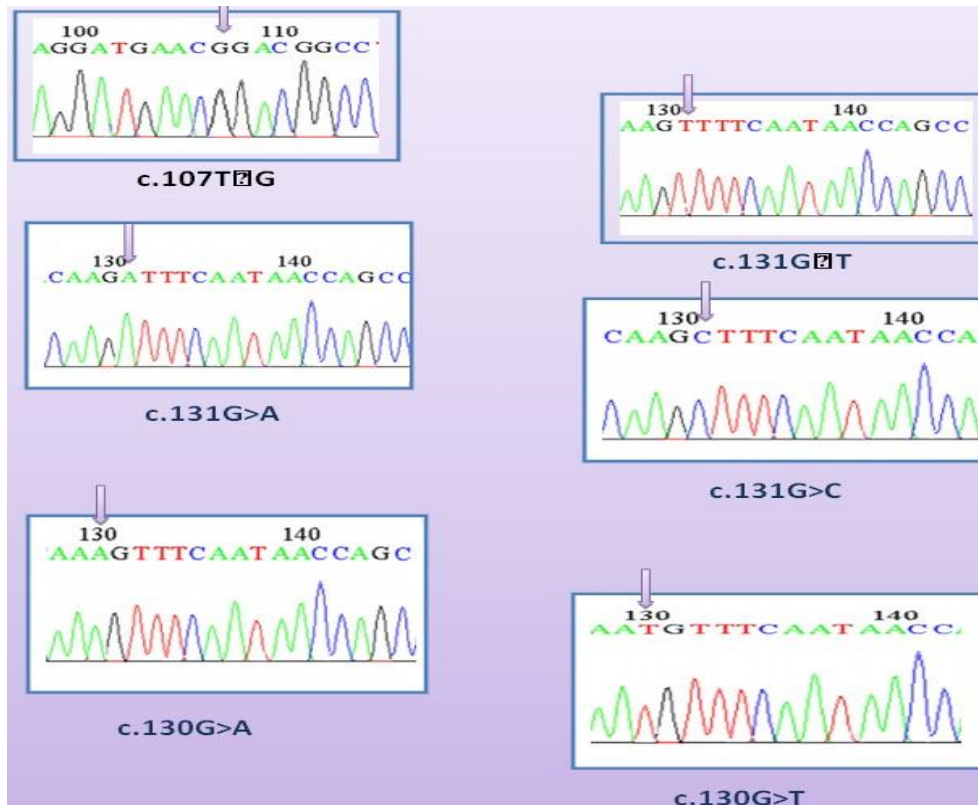


Fig. 1. MED12 genomic mutations.

**Table 1**

Clinicopathological features of 23 uterine tumors evaluated for MED12 exon 2 mutations.

Sample ID	Age at diagnosis	Number of fibroids	Size	Nucleotide change of MED12 exon 2
1	38	2	Anterior no size	Without mutation
2	41	2	1.3 x1.1 cm	c.107T>G
3	30	1	Fundal no size	c.131G>T
4	48	2	Inferior 4.5 x 3.7 cm	c.131G>A
5	50		Not reported	c.131G>C
6	42	3	5.0 x 3.5 cm	Without mutation
7	47	2	Posterior 4.3 x 2.8 cm	c.130G>A
8	52		Not reported	Without mutation
9	38	2	Anterior 4.3 x 4.1 cm	Without mutation
10	46	Multiple	Not reported	c.133_150del18
11	37	5	Fundal 6.5 x 3.2 cm	c.131G>A
12	35	1	Anterior no size	c.131G>T
13	41	2	1.3 x 1.3 cm	Without mutation
14	44		Not reported	Without mutation
15	53	1	Not reported	c.131G>A
16	48	1	Inferior 5.3 x 4.7 cm	c.130G>T
17	59	Multiple	Lateral no size	Without mutation
18	37	2	Anterior 4.2 x 3.9 cm	c.131G>A
19	46	1	Posterior no size	c.130G>A
20	56	2	Posterior no size	c.116_154del39
21	39	2	Fundal no size	Without mutation
22	42	1	Inferior 2.3 x 1.5 cm	c.131G>T
23	47	Multiple	Lateral no size	c.131G>A

Our recent study revealed very specific mutations in MED12 exon 2 in as many as 65% of uterine leiomyomas. We therefore hypothesised that these tumours might also harbor mutations in MED12. Uterine leiomyomas are benign smooth muscle tumors that can emanate from anywhere in the uterus and distort uterine anatomy and function. Leiomyomas are the leading cause of dysfunctional uterine bleeding and hysterectomies. The clonal origin of leiomyomas makes them genetically homogenous. However, karyotypes between individual leiomyomas differ, and abnormal karyotypes are identified in approximately 40% of cases (Rein et al., 1991; Canevari et al., 2005; Mäkinen et al., 2011).

Ethnicity is an important epidemiological risk factor for uterine leiomyomas in the general population. The effect of race on incidence and severity of fibroids is particularly significant. Several studies have reported a higher incidence of fibroids among Black women than other racial and ethnic groups including Caucasian, Hispanic, and Asian women (Marshall et al., 1997; Templeman et al., 2009; Kjerulff et al., 1996). The reasons for ethnic variation in uterine leiomyoma occurrence are unknown various possible causes for higher prevalence and greater symptom severity have been proposed. For example, the differences may be due to genes that confer increased risk for poor outcome. Uterine leiomyomas are hormonally.

Mäkinen et al. found that approximately 70% contained heterozygous somatic mutations that affect *MED12* on the X chromosome (Mäkinen et al., 2011). The mutated allele was either predominantly or exclusively expressed in affected tumors (Perot et al., 2012). Other studies confirmed these findings and established that mutations in *MED12* are also present in small subsets of other mesenchymal tumors of the uterus or in other tissues, although the uterine fibroid remains the most frequently affected tumor (Perot et al., 2012; Markowski et al., 2012).

Responsive tumors, and for instance, Asian woman have been reported to have a lower prevalence of oestrogen receptor- $\alpha$  PP variant than black women. The variant has been associated with an increased risk of uterine leiomyomas in both ethnicities (Al-Hendy and Salama, 2006).

To our knowledge, this is the first description of MED12 exon 2 mutation analysis in uterine leiomyomas From Iranian Tehran province women. The discovery of MED12 as an important target for driver mutations in 50–70% of unselected uterine leiomyomas has been an important step toward understanding the tumorigenesis of these extremely common lesions.

#### 4. Conclusion

Uterine leiomyoma is a benign tumor that rarely results in death. However, it remains quite common and is a main cause of infertility and hysterectomy.

Recurrent occurrence of MED12 mutations in exon 2 suggests a possibility that targeting of the mutation could be used in early diagnosis and nonsurgical treatment of uterine leiomyomas. The Mutations in exon 2 of the MED12 gene is related to an increased susceptibility to uterine leiomyoma, suggesting that this polymorphism does contribute to the development of this disease.

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**How to cite this article:** Ahmadi, A.R., Porrahim, H., Moghadasi, H., Najafi, N., Rakhshan, A., 2016. MED12 exon 2 mutations in uterine leiomyoma's in women patients of Tehran Province of Iran. *Scientific Journal of Biological Sciences*, 5(1), 103-108.

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