

# MED12 Exon 1 Mutational Screening in Iranian Patients with Uterine Leiomyoma

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## Abstract

**Background:** Uterine leiomyoma, also called fibroid, is a benign tumor that arises due to monoclonal transformation of myometrium, the smooth muscle cell layer of the uterus. Fibroids cause several complications including infertility, miscarriage, bleeding, pain, and dysmenorrhea. Recent studies have revealed the role of mutations in *MED12* gene exon 2 in various populations; however, the reported frequency of these mutations differs between reports. In addition, it is suggested that mutations in exon 1 may also play a role in leiomyoma. The aim of the present study was to screen for *MED12* exon 1 mutations in leiomyoma tissue samples of Iranian patients.

**Methods:** We performed mutational analysis of exon 1 and the flanking intronic regions using multi-temperature single-strand conformational polymorphism (MSSCP) and sequencing analyses in 120 uterine leiomyoma samples.

**Results:** No mutations were detected in exon 1 of *MED12* in our samples.

**Conclusions:** According to the literature and the present results, mutations in the *MED12* exon 1 are rare. However, we could not ignore the role of these mutations in developing leiomyoma.

**Keywords:** Exon 1, *MED12*, Mutation, Uterine leiomyoma.

## Introduction

Uterine leiomyoma (UL), also called fibroid, is one of the most prevalent women's health disorders worldwide. Greater than 10% of middle-aged women are diagnosed with UL (1). These benign tumors can cause several complications including pain, bleeding, and infertility, and are the most common cause for hysterectomies worldwide (2-4). Unfortunately, despite its associated health problems and high incidence, UL has no definite cure (5). To suggest an effective treatment, a more complete understanding of its molecular mechanism than is currently available is necessary. Studies from various ethnic groups have shown that the *MED12* protein (mediator complex subunit 12) (*MED12*) is mutated with high frequency in UL (6-9). Mediator

is a large macromolecular complex with functional versatility that contains at least 31 subunits. Mutations in *MED12* can disrupt the kinase activity of the mediator complex (9-11). Growing evidence suggests that mutations in exon 2 of the *MED12* gene (*MED12*) are the major cause of UL (6-9). According to the literature, the frequency of *MED12* exon 2 mutations varies from 52-80% between populations (11, 12). Kampjarvi et al. reported that mutations in *MED12* exon 1 also occur in UL samples (11). Recently, we screened 174 leiomyoma samples for *MED12* exon 2 mutations in Iranian patients and found that 31.07% of our samples had mutations in intron 1 and exon 2; this was the lowest reported frequency (7).

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Due to the low frequency for *MED12* exon 2 mutations in our samples and the results obtained from Kampjarvi *et al*, the aim of the present study was to investigate *MED12* exon 1 for possible mutations in UL samples that had no mutations in exon 2.

## Materials and methods

The present study was a descriptive study carried out at Shahid Beheshti University of Medical Sciences (SBMU). The study was approved by the Ethics Committee of the SBMU and informed consent was obtained from each patient. Totally, 174 UL samples were obtained after surgery from Iranian women and stored in RNA later solution. Genomic DNA was extracted from 100 mg of freshly frozen tissue using the M&D DNA extraction Kit (Shahid Beheshti University of Medical Sciences, Iran), according to the manufacturer's protocol. All samples were previously screened for *MED12* exon2 gene mutations, among which 120 UL samples that did not have exon2 mutations were included in the present study.

### Mutation analysis

To analyze mutations in *MED12* exon 1 and its flanking intron regions, genomic DNA was amplified by PCR using specific primers (*MED12* F: 5'-GCCGTCCTCTCAACCACC-3'; *MED12* R: 5'CGTCAGTTCATCCTCCTTCTGT-3'; product size of 316 bp). Each PCR contained 100 ng of DNA, 10  $\mu$ l of Taq DNA Polymerase 2X Master Mix Red (Amplicon, Denmark), and 10 pmol/ $\mu$ l of each primer in a final volume of 25  $\mu$ l. Amplifications were performed on a FlexCycler (Analytik Jena, Germany) with the following program: initial denaturation at 94 °C for 5 min and a subsequent series of 35 cycles of 94 °C for 30 sec (denaturation), 61 °C for 45 sec (annealing), and 72 °C for 30 sec (extension). A final extension was performed at 72 °C for 5 min.

The PCR products were then subjected to multi-temperature single-strand conformation polymorphism analysis (MSSCP) on an 8% nondenaturing polyacrylamide gel according to the standard protocol (12). To confirm the MSSCP data, samples with altered banding patterns and ten percent of the samples with

normal banding patterns were sequenced on an ABI 3730XL automated DNA sequencer (Macrogen, Seoul, Korea).

## Results

In this study *MED12* exon 1 was examined in 120 UL samples. Our patients ages ranged from 29 to 60 years (mean $\pm$ SD=38.15 $\pm$ 7.46 years). The tumor sizes ranged from 0.7–22 cm in diameter (5.18 $\pm$ 3.36 cm). Three different types of tumors were seen; these included intramural, subserous, and submucous fibroids with frequencies of 50, 35, and 15% respectively. Using the MSSCP method, no differences were seen between the banding patterns of the UL and normal uterine samples. We concluded that no UL samples had mutations in *MED12* exon 1.

## Discussion

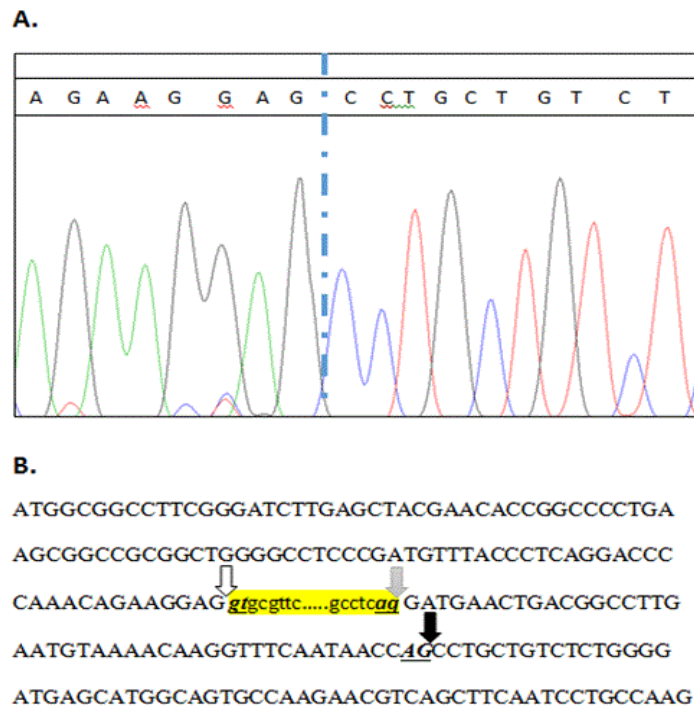
The purpose of the present study was to investigate *MED12* exon 1 for mutations and their potential roles in the development of UL. None of our UL samples had mutations in *MED12* exon 1. According to the literature, *MED12* mutations in UL are mainly concentrated in exon 2. Kampjarvi *et al* analyzed *MED12* exon 1 in 611 tumor samples, including those from ULs, extrauterine leiomyomas, endometrial polyps, uterine leiomyosarcomas, other sarcomas, and colon carcinomas. They reported five *MED12* exon 1 mutations, all in ULs. All the mutations were in-frame indels, including c.82\_99del18, c.76\_91del16insG, c.84\_98del15 and c.79\_99del21 (11). Our result agrees with previous studies that included analysis of all *MED12* exons using whole exome sequencing technique (13-17).

Mediator is large macromolecular complex with versatile functions containing at least 31 subunits. The complex is arranged in four modules, including head, middle, tail, and kinase modules. The latter section contains *MED12*, *MED13*, *CDK8*, and *cyclin C* (6). The mediator complex is a pivotal coactivator of transcription. The precise mechanisms by which mediator regulates Pol II activity remain poorly understood, but it is well established that mediator, with its kinase activity, can regulate

phosphorylation of the RNA polymerase II C-terminal domain. Hence, any alteration in *MED12* disrupting the kinase module can have adverse effects on its regulatory functions. Both exons 1 and 2 encode the cyclin C binding domain of *MED12*. Hence, mutations in these exons disrupt *MED12* cyclin C binding and result in reduced affinity for cyclin C-CDK8 and loss of mediator-associated CDK function (9-11). Therefore, the proper exon sequence is vital for the protein's function.

The second point to address is the possible

effect of mutations located in intron 1 near exon 2 or that span the intron 1-exon 2 junction. In our previous study, we reported a 34-nucleotide deletion in one sample that spans the intron 1-exon 2 junction (g.5812\_5845del34) and concluded that this alteration may affect the gene transcript splicing (7). Further analysis of the cDNA sequence revealed that this mutation activates a cryptic acceptor splice site inside the *MED12* exon 2 region that expands the deleted region up to 51 nucleotides in the cDNA sequence (Fig. 1).



**Fig. 1.** Nucleotide Sequence of the New Cryptic Splice Site

A. The cDNA sequence of the g.5812\_5845del34 mutation. The dashed line indicates the exon 1-exon 2 junction at the cDNA level.

B. The sequence of exon 1, exon 2, and intron 1. The white arrow indicates the exon 1-intron1 boundary (splice donor site). The gray arrow indicates the intron 1-exon 2 boundary (splice acceptor site). The black arrow indicates the new splice acceptor site.

This alteration results in cDNA and protein sequences c. 100-6\_144del51 and p. D34\_Q48del, respectively. This observation is interesting since it shows that mutations in regions surrounding the intron-exon boundary may activate the cryptic acceptor splice site; therefore, the number of deleted bases will differ between genomic DNA and RNA after splicing.

Few mutations in *MED12* exon 1 have been reported, however, similar to exon 2 mutations, exon 1 mutations can lead to loss of the mediator-related kinase activity by disrupting the interaction between

*MED12* and cyclin C. In conclusion, no mutation was detected in exon 1 of *MED12* in our studied samples, and it seems that the frequency of these mutations is rare in uterine leiomyomas.

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