European Journal of Human Genetics (2013) 21, 1300–1303

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SHORT REPORT

MED12 exon 2 mutations in histopathological uterine leiomyoma variants

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Uterine leiomyomas, or fibroids, are the most common human tumors. Based on histopathology, they can be divided into common leiomyomas and various relatively rare subtypes that mimic malignancy in one or more aspects. Recently, we showed that exon 2 of *mediator complex subunit 12* (*MED12*) is mutated in up to 70% of common fibroids. To investigate the frequency of *MED12* exon 2 mutations in histopathological uterine leiomyoma variants, we screened altogether 206 lesions, including 69 histopathologically common leiomyomas, 59 cellular (23 cellular and 36 highly cellular), 18 atypical and 26 mitotically active leiomyomas, as well as 34 uterine fibroid samples from 14 hereditary leiomyomatosis and renal cell cancer patients with a heterozygous germ line mutation in *fumarate hydratase* (*FH*). The uterine leiomyoma variants harbored *MED12* exon 2 mutations significantly less frequently than common leiomyomas ($P = 2.93 \times 10^{-8}$). In all, 6 mutations were detected among cellular fibroids (6/67; 8.96%), 3 among atypical fibroids (3/18; 16.67%) and 10 among mitotically active fibroids (10/26; 38.46%). Only mitotically active fibroids displayed a mutation frequency that was not statistically different from common leiomyomas ($P = 0.11$). Three *MED12* exon 2 mutations were detected among 34 tumors with a heterozygous germ line *FH* mutation ($P = 5.28 \times 10^{-7}$). None of these tumors displayed biallelic inactivation of *FH*. Our results suggest that *MED12* mutation positivity is a key characteristic of common leiomyomas. Cellular and atypical fibroids, in particular, may arise through different molecular mechanisms. The results also propose that *MED12* and biallelic *FH* mutations may be mutually exclusive.
European Journal of Human Genetics (2013) **21,** 1300–1303; doi:10.1038/ejhg.2013.33; published online 27 February 2013

Keywords: uterine fibroid; uterine leiomyoma variants; *MED12*; *FH*

INTRODUCTION

Uterine leiomyomas, also known as fibroids, are benign tumors that occur in nearly 70% of women by the age of 50 years.[1] Regardless of their benign nature, fibroids can cause a variety of health problems, such as abnormal menstrual bleeding and infertility.[2] Uterine leiomyomas are the most common cause for hysterectomy and pose a considerable socioeconomic burden.[3,4] Hormonal factors, such as estrogen and progesterone, are known to have a role in the growth of leiomyomas and the lesions may regress when hormone levels decrease.[5] Based on histopathology, fibroids can be classified in common leiomyomas and several relatively rare subtypes, such as cellular, atypical, mitotically active, epitheloid and myxoid fibroids. These histopathological variants account for $<5\%$ of all uterine leiomyomas. Although these variants mimic malignancy in one or more aspects,[6] their behavior is benign.

Fibroids also occur in the context of hereditary leiomyomatosis and renal cell cancer (HLRCC, OMIM # 150800). HLRCC is a tumor susceptibility syndrome predisposing to cutaneous and uterine leiomyomatosis, and in some families also to renal cell cancer.[7,8] It is caused by heterozygous germ line mutations in *fumarate hydratase* (*FH*), which encodes the citric acid cycle enzyme fumarase.[9] Uterine leiomyomas associated with this syndrome display loss of the normal *FH* allele, occur at young age and require treatment more often than sporadic uterine leiomyomas.[10] The role of *FH* in sporadic uterine leiomyoma tumorigenesis has previously been investigated and biallelic inactivation of *FH*, the hallmark of HLRCC leiomyomatosis, has been detected in only 1.3% of the tumors.[11,12]

Recently, we discovered specific mutations in exon 2 of *mediator complex subunit 12* (*MED12*) in as many as 70% of the 225 unselected uterine leiomyomas studied.[13] The majority represented missense mutations affecting a single codon glutamine 44. MED12 is part of a multiprotein complex called Mediator, which participates in regulation of global as well as gene-specific transcription.[14] MED12 forms together with MED13, CDK8 and cyclin C a Mediator subcomplex known as the CDK8 module. This module interacts with the main Mediator complex, but functions outside the complex have also been proposed.[15] MED12 is a key regulator of the kinase activity of CDK8 module, and the protein directly interacts with multiple transcription factors, such as β-catenin.[14–16]

These recent advances may provide tools for molecular classification of uterine fibroids. To examine the prevalence of *MED12* exon 2 mutations in various uterine leiomyoma subtypes, we first made a significant effort to derive an appropriate sample set to allow addressing this question. We then screened altogether 206 uterine leiomyoma samples representing common leiomyomas, the most common histopathological variants of leiomyoma, and fibroids from HLRCC patients for the *MED12* mutations.

MATERIALS AND METHODS

Research permits

All the samples used in this study were derived according to Finnish laws and regulations, either after informed consent or, if anonymized, after authorization from the director of the respective health-care unit. The study has been

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Received 11 October 2012; revised 18 January 2013; accepted 30 January 2013; published online 27 February 2013

approved by the appropriate ethics review board of Helsinki University Central Hospital, Finland.

Archival tissue samples in *MED12* exon 2 mutation analysis

A series of 172 anonymous archival formalin-fixed paraffin-embedded (FFPE) uterine leiomyoma samples was collected at the Department of Pathology, Hospital District of Helsinki and Uusimaa, Finland. The series consisted of 69 common leiomyomas, as well as 59 cellular (23 cellular and 36 highly cellular), 18 atypical and 26 mitotically active leiomyomas (8 lesions showing simultaneously increased cellularity). The representatives of each subtype are shown in Figure 1. Eight mitotically active leiomyomas with increased cellularity were included to both mitotically active and cellular leiomyoma groups for statistical testing. Also a set of 34 uterine fibroid samples from 14 HLRCC patients with known *FH* mutation status[17] was available for *MED12* exon 2 mutation screening. None of the samples have been previously published in respect of *MED12* mutations.

Histopathological evaluation of tissue samples

Histopathological assessment of uterine leiomyoma samples was performed by a pathologist (RB). FFPE blocks and ice blocks from fresh frozen tissue samples harboring primarily tumor tissue were selected, sectioned on a microtome/cryotome to 5 μm in thickness and stained with hematoxylin–eosin (HE) according to standard procedures. HE-stained sections from each specimen were reviewed and the tumors were classified into common, cellular, atypical and mitotically active leiomyomas according to the WHO criteria.[6] The number of mitotic figures per 10 high-power fields, the degree of cellularity (normal, cellular and highly cellular) and severity of nuclear atypia (0–3) were recorded for each uterine leiomyoma. See Supplementary Tables S1 and S2 for detailed histopathological data.

MED12 exon 2 mutation screening

MED12 mutation screening was carried out by direct sequencing. Genomic DNA from the FFPE samples was extracted either with NucleoSpin FFPE DNA Kit or with NucleoSpin FFPE RNA/DNA Kit (Macherey-Nagel, Düren, Germany). Oligonucleotide primers were designed with Primer3[18] using Ensembl release 62 as the reference. The primer sequences in the 5′ to 3′ direction are GCCCTTTCACCTTGTTCCTT (forward) and AAGCTGACGT TCTTGGCACT (reverse) covering all the observed mutation hotspots in *MED12* exon 2 and intron 1. PCR analyses were performed in duplicate to ensure accuracy, with two different amounts of DNA: 15 and 25 ng. PCR products were sequenced utilizing Big Dye Terminator v.3.1 Kit (Applied Biosystems, Foster City, CA, USA) on an ABI3730 Automatic DNA Sequencer according to the manufacturer's instructions. The sequence graphs were analyzed both manually and with Mutation Surveyor-software (Softgenetics, State College, PA, USA).

Detection of *FH* loss of heterozygosity (LOH)

To assess LOH, tumor DNA from patients with a germ line *FH* mutation was sequenced, as described above. Three different sets of primers were utilized, one for each mutation: 5′-TTTGTTTTTGTTGCCTCTGATTT-3′ and 5′-GGA TTTTGCATCAAGAGCATC-3′ (c.587A>G, p.H196R), 5′-TGCTGCTGCAAT AGAAGTTCA-3′ and 5′-CCTGCCCAAGAGTAAGTGGA-3′ (c.671_672delAG, p.E224fs), and 5′-GCTCTGGTTGAGCTCAGTGG-3′ and 5′-GGACCTAGTCA AGTTTTAGCTCCA-3′ (c.1027C>T, p.R343X). For each sample, five parallel PCR reactions were carried out to ensure accuracy. LOH was determined manually by comparing the heights of wild-type and mutant allele peaks. When the height of wild-type allele peak was repeatedly significantly lower than the height of mutant allele peak, LOH was scored.

Statistical analyses

Statistical analyses were performed using R software, version 2.14.0 (R Foundation for Statistical Computing, Vienna, Austria). Differences between the frequencies of *MED12* exon 2 mutations in clinical uterine leiomyoma subtypes and common uterine leiomyomas were calculated with Fisher's exact test. All *P*-values are two-sided and *P*-value <0.05 was considered statistically significant.

RESULTS

MED12 exon 2 mutation statuses were determined for 103 uterine leiomyoma variants and 69 common leiomyomas. Altogether 18 out of 103 histopathological variants (17.48%) harbored a mutation in *MED12* exon 2 (Table 1). Ten mutations were observed among mitotically active fibroids (10/26; 38.46%), three among atypical fibroids (3/18; 16.67%) and six among cellular fibroids (6/67; 8.96%). Of the 42 highly cellular fibroids, only two carried a *MED12* mutation (2/42; 4.76%). In contrast, the majority of common leiomyomas (41/69; 59.42%) displayed a *MED12* mutation. We observed altogether 59 *MED12* exon 2 mutations: 39 missense mutations in codon 44, three in codon 36 and one in codon 43, as well as 15 insertion–deletion type mutations (see Supplementary Tables S1 and S3 for more detailed information of *MED12* statuses). The majority of these mutations have previously been reported.[13] In addition, one novel missense mutation c.122T>A, p.V41E was detected in a common leiomyoma.

Overall, the histopathological variants of uterine leiomyoma harbored *MED12* exon 2 mutations significantly less frequently

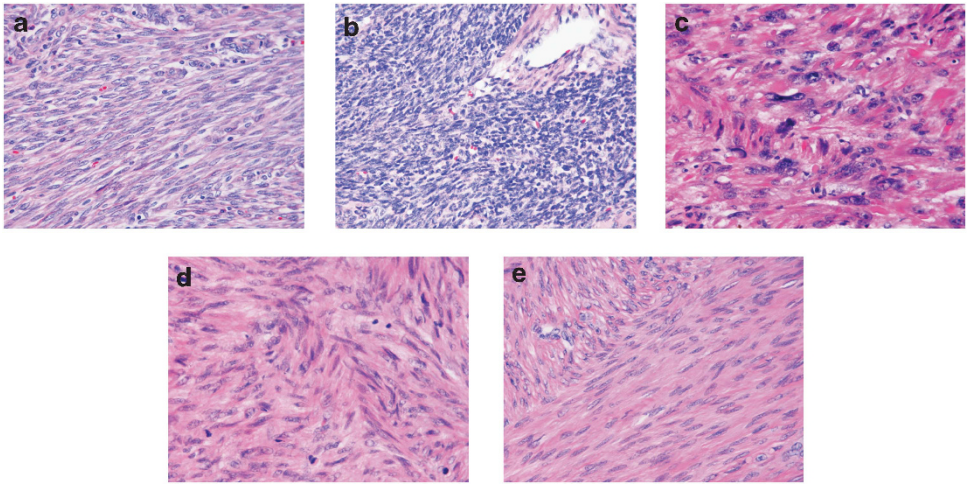

Figure 1 Histopathological variants of uterine leiomyoma. (**a**) Cellular leiomyoma. (**b**) Highly cellular leiomyoma. (**c**) Atypical leiomyoma. (**d**) Mitotically active leiomyoma. (**e**) Common leiomyoma. HE-stainings are shown with $\times 40$ magnification.

Table 1 *MED12* exon 2 mutations in clinical uterine leiomyoma subtypes

Leiomyoma subtype	*Total*	*Mutation positive lesions*		*P-value*	*OR (95% CI)*
Common leiomyoma	69	41	59.42%		
Leiomyoma variants	103	18	17.48%	2.93×10^{-8}	6.82 (3.26–14.83)
Cellular leiomyoma	67	6	8.96%	2.49×10^{-10}	14.55 (5.34–46.90)
Cellular (+)	25	4	16.00%	1.82×10^{-4}	7.52 (2.21–33.42)
Highly cellular (++)	42	2	4.76%	1.51×10^{-9}	28.45 (6.49–260.56)
Atypical leiomyoma	18	3	16.67%	1.40×10^{-3}	7.16 (1.79–42.15)
Mitotically active leiomyoma	26	10	38.46%	1.05×10^{-1}	2.32 (0.85–6.64)
Leiomyomas from HLRCC-patients	34	3	8.82%	5.28×10^{-7}	14.74 (4.01–82.59)

Eight tumors were included in both mitotically active and cellular leiomyomas.

than common leiomyomas ($P = 2.93 \times 10^{-8}$; OR, 6.82; 95% CI, 3.26–14.83; Table 1). Only mitotically active fibroids displayed a *MED12* mutation frequency that was not statistically different from the common leiomyomas ($P = 0.11$; OR, 2.32; 95% CI, 0.85–6.64). The lowest mutation frequency was observed in highly cellular fibroids ($P = 1.51 \times 10^{-9}$; OR, 28.45; 95% CI, 6.49–260.56).

A total of 34 fibroids from 14 HLRCC patients with a known germ line *FH* mutation were screened for *MED12* exon 2. Only three cases (8.82%) displayed a mutation in *MED12*, which was significantly less frequently compared with common leiomyomas ($P = 5.28 \times 10^{-7}$) (Table 1, see Supplementary Table S2 for more detailed information). In our previous studies, biallelic inactivation of *FH* has been shown in 14 out of these 34 HLRCC fibroids. The remaining lesions were assessed for LOH, as a marker of *FH* driven tumorigenesis and altogether 21 fibroids (61.76%) exhibited clear biallelic *FH* inactivation. LOH was not detected in any of the *MED12* mutation positive lesions (see Supplementary Table S2 for more detailed information).

DISCUSSION

The discovery of *MED12* as an important target for driver mutations in 50–70% of unselected uterine leiomyomas[13,19–22] has been an important step toward understanding the tumorigenesis of these extremely common lesions. Recently, *MED12* exon 2 mutations have also been detected in uterine leiomyosarcomas and colorectal cancer.[22–24] Although these mutations are not restricted to fibroids, the majority seem to be characteristic to uterine malignancies. So far, the relevance of the mutations for molecular classification of fibroids has remained poorly understood. To investigate the association between *MED12* mutation positivity and different histopathological variants of uterine leiomyoma, as well as tumors with a germ line *FH* mutation, we screened *MED12* exon 2 in altogether 206 uterine fibroid samples.

MED12 mutations were significantly less frequent among unusual histopathological variants of uterine leiomyoma as compared with common leiomyomas ($P = 2.93 \times 10^{-8}$). Only 16.7% of atypical leiomyomas harbored a *MED12* exon 2 mutation. Atypical lesions exhibit nuclear pleomorphism, but lack tumor necrosis and mitotic figures, which are other markers of malignancy. These tumors are often also cellular and like common leiomyomas, their course is benign. Cellular leiomyomas are defined as fibroids with cellularity that is greater than in the normal myometrium. In the utmost form, the cells lack spindle-shaped form and fascicular arrangement typical to leiomyomas, and these tumors have been designated as highly cellular leiomyomas. *MED12* exon 2 mutations were uncommon in cellular leiomyomas, and particularly rare in highly cellular leiomyomas ($P = 1.51 \times 10^{-9}$). The rare occurrence of *MED12* exon 2 mutations in this histopathological subtype suggests a distinct molecular pathogenesis. The difference between the frequencies of *MED12* mutation positive lesions in mitotically active and common leiomyomas did not reach significance ($P = 0.11$). This is in keeping with the concept that hormonal and other external factors, not directly related to the tumors themselves, are important for this phenotype.[25]

Uterine leiomyomas associated with HLRCC have been suggested to display increased cellularity, atypia and occasional mitoses, the same histopathological features that characterize uterine leiomyoma variants.[26] In addition, they have been proposed to exhibit other distinct features, such as orangeophilic nucleoli surrounded by a perinucleolar halo.[27] Previous literature has suggested that *FH* inactivation associates with larger, more numerous, and symptomatic tumors, although malignant transformation is rare.[26,28] Only three *MED12* exon 2 mutations were observed among 34 fibroids from HLRCC patients ($P = 5.28 \times 10^{-7}$). Unlike in typical HLRCC lesions[8,11] and while biallelic inactivation of *FH* was observed in the majority of the studied HLRCC fibroids, loss of the remaining normal *FH* allele was not detected in the three *MED12* mutant tumors. These three cases may represent incidental lesions not related to the germ line *FH* mutation. Thus, we did not find any tumors that had both a *MED12* mutation as well as biallelic *FH* inactivation, suggesting that *MED12* and *FH* mutations may represent two different pathways for leiomyomagenesis. Recent study by Markowski *et al.* showed that *MED12* mutations were also absent in tumors harboring the most common cytogenetic rearrangement, a translocation involving chromosome bands 12q15 and 14q24.[20,29] This rearrangement is known to upregulate *high mobility group AT-hook 2* (*HMGA2*) gene at 12q15,[30,31] and thus might provide a third pathway for leiomyomagenesis.

In conclusion, our results indicate that *MED12* exon 2 mutations are characteristic to common leiomyomas. We also suggest that *MED12* and *FH* mutations might be mutually exclusive. Before significant progress toward targeted therapies on fibroids can be made, the events leading to genesis of these frequent lesions need to be understood in great detail. The emerging molecular classification should provide valuable tools for accurate stratification of fibroids, as well as clues to the exact molecular mechanisms underlying this tumor type.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Sini Nieminen, Inga-Lill Svedberg and Mairi Kuris for excellent technical assistance. This study was supported by the Academy of Finland (Center of Excellence in Cancer Genetics Research, grant 250345 and personal

grant 260370 to PV), the Sigrid Jusélius Foundation, and the Cancer Society of Finland, as well as Emil Aaltonen Foundation, Orion-Farmos Research Foundation and K Albin Johansson Foundation (personal grants to NM).

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)