Systematic molecular and clinical analysis of uterine leiomyomas from fertile-aged women undergoing myomectomy

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Systematic molecular and clinical analysis of uterine leiomyomas from fertile-aged women undergoing myomectomy

Running title: Uterine leiomyomas from myomectomies

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Abstract

Study question: What are the distributions and associated clinical characteristics of mediator complex subunit 12 *(MED12)*, high mobility group AT-hook 2 (HMGA2), and fumarate hydratase (FH) aberrations in uterine leiomyomas from fertile-aged myomectomy patients?

Summary answer: These driver mutations account for the majority (83%) of tumours in fertile aged patients.

What is known already: Alterations affecting *MED12*, HMGA2, and FH account for 80−90% of uterine leiomyomas from middle-aged hysterectomy patients, while the molecular background of tumours from young myomectomy patients has not been systematically studied.

Study design, size, duration: A retrospective series of 361 archival uterine leiomyoma samples from 234 women aged ≤45 years undergoing myomectomy in 2009–2014 was examined. Associations between the molecular data and detailed clinical information of the patients and tumours were analysed.

Participants/materials, setting, methods: DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples and *MED12* exons 1 and 2 were sequenced to identify mutations. Level of HMGA2 expression was evaluated by immunohistochemistry. Biallelic fumarate hydratase *(FH)* inactivation was analysed with 2-succinylcysteine staining, which is an indirect method of assessing FH deficiency. All patients' medical histories were reviewed, and clinical information of patients and tumours was combined with molecular data.

Main results and the role of chance: The median age at operation was 34 years. The majority (58%) of patients were operated on for a single leiomyoma. Known driver mutations were identified in 83% of tumours (71% *MED12*; 9% HMGA2; 3% FH). In solitary leiomyomas, the *MED12* mutation frequency was only 43%, and 29% were wild-type for all driver alterations. *MED12* mutations were associated with multiple tumours, smaller tumour size, and subserosal location.

Limitations, reasons for caution: Although comprehensive, the study is retrospective in nature and all samples had been collected for routine diagnostic purposes. The use of paraffin-embedded samples and immunohistochemistry may have led to an underestimation of mutations. Due to the limited sample size and rarity of especially FH-deficient leiomyomas, the data are partly descriptive.

Wider implications of the findings: The contribution of driver mutations in leiomyomas from young myomectomy patients is comparable to tumours obtained from hysterectomies of mostly middle-aged women. Our results support the earlier findings that *MED12* mutations are associated with multiple tumours, smaller tumour size and subserosal location. The study emphasizes the distinct molecular background of solitary leiomyomas, and more research is needed to clarify the underlying causes of the notable proportion of wild-type leiomyomas.

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Keywords

Uterine leiomyoma, myomectomy, mediator complex subunit 12 (*MED12*), high mobility group AT-hook 2 (HMGA2), fumarate hydratase (FH)

Introduction

Uterine leiomyomas are common, benign smooth muscle tumours with a prevalence as high as 70-80% by the age of 50 years (Baird et al., 2003). The majority can be classified as conventional tumours, whereas ~10% belong to one of several histological variants such as mitotically active, cellular, and epithelioid leiomyoma, and leiomyoma with bizarre nuclei (Oliva et al., 2014). Most leiomyomas are asymptomatic, but at least 20% of women with these tumours suffer from symptoms requiring treatment such as abnormal uterine bleeding, pelvic pressure, urinary complaints, bowel dysfunction, and even infertility (Vilos et al., 2015; Klatsky et al., 2008). Hysterectomy is a definitive treatment, while myomectomy is a surgical option for patients who wish to preserve their uterus.

Genetic analyses have revealed several different pathogenic pathways in the development of leiomyomas (reviewed in Mehine et al., 2014). Specific mutations in mediator complex subunit 12 *(MED12)* occur in 50-90% of leiomyomas depending on the ethnicity of the patients. Mediator complex subunit 12 is part of the multiprotein complex Mediator, which is an evolutionarily conserved regulator of RNA polymerase II -mediated transcription (Croce and Chibon 2015). *MED12* mutations lead to the uncoupling of Cyclin C and CDK8/19 from the core Mediator, loss of Mediator associated CDK kinase activity, and a unique global gene expression pattern (Mehine et al., 2013; Turunen et al., 2014; Kämpjärvi et al., 2014). In addition to uterine leiomyomas, *MED12* mutations have been reported in other female hormone-dependent tumours such as breast fibroadenomas (Chang et al., 2020), phyllodes tumours, and uterine adenomyomas (Heikkinen et al., 2018). Roughly 10% of leiomyomas show high mobility group AT-hook 2 (HMGA2) overexpression. HMGA2 is a non-histone chromatin-binding protein that is normally expressed only in undifferentiated mesenchymal tissue. Overexpression of HMGA2 in well differentiated mesenchymal cells may lead to tumourigenesis by disturbing cell proliferation, cell cycle regulation, DNA damage response, and apoptosis (Unachukwu et al., 2020). Fumarate hydratase (FH) deficiency in leiomyomas is relatively

rare, but particularly important due to the association with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) syndrome. HLRCC is caused by a germline mutation in fumarate hydratase *(FH*), which predisposes also to cutaneous leiomyomas and type 2 papillary renal cell carcinoma (Launonen et al., 2001; Tomlinson et al., 2002). *FH* is a tumor suppressor gene, and the enzyme fumarate hydratase acts in the tricarboxylic acid cycle, which is essential for the metabolism of cells. *MED12*, HMGA2, and FH aberrations have been reported as mutually exclusive in leiomyomas (Markowski et al., 2012; Bertsch et al., 2014; Kämpjärvi et al., 2016; Mäkinen et al., 2017; Mehine et al., 2013), but recently *HMGA2* overexpression at RNA level was noted also in *MED12*-positive tumours (Galindo et al., 2018; Mello et al., 2018).

Based on earlier studies, the three aforementioned driver alterations account for 80-90% of uterine leiomyomas (Mehine et al., 2014). Most previous studies, however, have analysed samples obtained through hysterectomy, thus concerning primarily women well over 40 years. Leiomyomas occurring in younger patients –women of fertile age undergoing myomectomy– are significantly less studied. The primary aim of this study was to determine the distribution of *MED12*, HMGA2, and FH aberrations in leiomyomas from fertile-aged myomectomy patients, and to identify associations between molecular and clinical characteristics.

Materials and Methods

Ethical approval

The study was approved by the appropriate ethics review board of the Hospital District of Helsinki and Uusimaa, Finland (24/13/03/03/2015) and carried out in accordance with the Declaration of Helsinki. All patients were contacted by regular mail before initiating the study; 62% (155/250) were reached and all but one gave their informed consent; the one patient who declined was omitted from

the study. Permission to complement the patient series was subsequently obtained from the National Supervisory Authority for Welfare and Health (Valvira; 602/06.01.03.01/2016).

Patient samples

The patient series is retrospective and includes women aged 17–45 years who have undergone an elective myomectomy at Helsinki University Hospital, Finland, during 2009–2014. Patients were identified based on the NOMESCO Classification of Surgical Procedures´ codes (Ree et al., 2009) for myomectomy (LCB10), and laparoscopic myomectomy (LCB11). Routine pathology reports were reviewed to confirm the leiomyoma diagnosis and to exclude other conditions such as adenomyomas. Archival formalin-fixed paraffin-embedded (FFPE) leiomyoma samples were collected at the Department of Pathology, Helsinki University Hospital. A pathologist specialized in gynaecological tumours (AP) re-evaluated haematoxylin-eosin -stained histological tissue samples that were initially diagnosed as other than conventional leiomyomas and classified them according to the 2014 WHO classification (Oliva et al., 2014). Patients' medical history, including a self-report questionnaire specific for gynaecologic history, was reviewed. The flow chart of the inclusion of patients and tumour samples is shown in Figure 1.

Tissue microarray construction

Tissue microarrays were constructed utilizing the FFPE blocks. Four 0.8 mm cores from the representative areas defined by the pathologist (AP) were punched into an empty paraffin block using a manual tissue arrayer (MTA-I, Beecher Instruments, Sun Prairie, WI, USA). Myometrium samples were included in each tissue microarray as normal tissue controls.

Immunohistochemistry

Biallelic *FH* inactivation was analysed with 2-succinylcysteine (2SC) staining, which is an indirect method of perceiving FH deficiency (Bardella et al., 2011). Lack of functional FH causes accumulation of fumarate, which in turn leads to elevated levels of succinated (2SC-modified) proteins recognized by an anti-2SC antibody. Immunostainings for 2SC-modified proteins and HMGA2 were performed on 5 µm tissue microarray sections using an anti-2SC antibody (1:1000; crb2005017, Discovery Antibodies, Cambridge Research Biochemicals, Billingham, Cleveland, UK) and an anti-HMGA2 antibody (1:2000; 59170AP, Biocheck Inc., Foster City, CA, USA). Heat-induced antigen retrieval in a microwave oven was followed by endogenous peroxidase blocking and overnight primary antibody incubation at 4 °C. Immunohistochemical staining for HMGA2 and 2SC was visualized by BrightVision system (Immunologic, Duiven, Netherlands) and DAB Quanto system (Thermo Fisher Scientific, Waltham, MA, USA). Samples showing aberrant staining at tissue microarray were further validated in a separate staining using whole tissue sections. Each set of staining included a positive control, and normal myometrium tissue was used as a negative control.

Visual scoring was performed by an experienced pathologist specialized in gynaecological tumours (AP). The scoring system contained four classes based on the fraction of positive cells: - = fully negative, (+) = single cell positivity, + = low heterogeneous positivity, ++ = diffuse (> 50% of the tumoral cells) positivity. Samples showing diffuse positivity were interpreted as positive. For HMGA2, only nuclear labelling was evaluated.

DNA extraction and mutation screening

Genomic DNA was extracted from seven 10 µm FFPE tissue sections or from six 0.8 mm cores if the amount of representative leiomyoma tissue in the FFPE block was limited. DNA was extracted with ReliaPrep FFPE gDNA Miniprep System (Promega, Madison, WI, USA), NucleoSpin DNA FFPE XS kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), or standard phenol-chloroform

method. Sequencing of *MED12* exons 1 and 2 and the coding region of *FH* in samples showing 2SC positivity was performed at the Institute of Molecular Medicine Finland, Helsinki, Finland, using Applied Biosystems ABI3730 Automatic DNA Sequencer. Details of the protocols and primers have been previously described (Kämpjärvi et al., 2014; Kämpjärvi et al., 2016). Electropherograms were analysed using Mutation Surveyor software (SoftGenetics, State College, PA, USA) and visual inspection.

Statistical methods

All statistical analyses were run in SPSS (IBM Corp., released 2017. IBM SPSS Statistics for Windows, version 25.0. Armonk, NY, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were exploited to check for normality of distribution. Median with range is presented for continuous variables that were not normally distributed. Independent observations assumption was applied to the data concerning patients. As several patients had had more than one tumour removed, data concerning individual leiomyomas were treated as non-independent observations. To account for possible correlation of observations, generalized estimating equations model with the logit link function was used to compare *MED12* frequency in solitary and multiple leiomyomas.

Due to non-normal distribution of variables, Kruskal-Wallis test was used to analyse continuous variables, followed by applicable pairwise comparison. Chi-square and Fisher´s exact test were used for comparison of categorical variables. If a statistically significant difference between groups was observed, multinomial logistic regression was performed. Two-sided p-values < 0.05 were considered statistically significant. For pairwise comparisons of continuous variables, significant values were adjusted by Bonferroni correction. Odds ratios (ORs) are reported with 95% confidence intervals (CIs).

Results

Altogether 234 myomectomy patients and 361 uterine leiomyomas were included in the study. Median age at operation was 34 years, and median BMI was 23. Of the patients, 177 (76%) were Finnish (white Caucasians) and 21 (9%) were of African descent. The majority (193; 82%) of patients reported themselves to be non-smokers. One hundred fifty-three patients (65%) were nulliparous, and 57 patients (24%) had a history of infertility, which was defined as an inability to conceive after 12 months of unprotected intercourse. A small subgroup of patients (15; 6.4%) had had a previous myomectomy. Gonadotrophin- releasing hormone agonists had been administered preoperatively for seven patients (3.0%) and selective progesterone receptor modulators for eight patients (3.4%). Detailed information on patient characteristics is presented in Table I.

The majority of patients (136; 58%) were operated on for a single leiomyoma, while 42 (18%) had two, 19 (8%) had three, and 16 (7%) had four leiomyomas removed. The remaining 21 patients had five or more leiomyomas (the range extended to 13 tumours) removed in one operation. Myomectomy was performed via laparotomy for 119 patients (51%), while 115 patients (49%) had laparoscopic myomectomy. Morcellation was used in 94 (82%) of the laparoscopic myomectomies.

Known driver alterations were detected in altogether 298/361 samples (83%). 255 samples (71%) harboured a mutation in *MED12*. In all but four cases, the mutation was in exon 2 and missense mutations affecting the hotspot codon 44 accounted for 176 (69%) of the *MED12* mutations detected. Exon 1 in-frame deletions were seen in four leiomyomas. All *MED12* mutations were heterozygous. Overexpression of HMGA2 was observed in 32/361 leiomyomas (9%), 11 (3%) showed positive 2SC staining indicating biallelic *FH* inactivation, and 63 (17%) were wild-type for all studied alterations (Fig. 2a). Detailed information on mutations is presented in Supplementary Table I.

Mutation frequencies in relation to the number of leiomyomas removed are shown in Figure 2b. In solitary tumours, the *MED12* mutation frequency was 43%, rising to over 80% in multiple leiomyomas. Generalized estimating equations model showed that the *MED12* mutation frequency was significantly higher in multiple leiomyomas than in solitary tumours ($p < 0.001$; OR 2.25, 95% CI 1.70–2.79). HMGA2 overexpression was seen in 21% of solitary leiomyomas; the frequency was low (up to 7%) in multiple leiomyomas. All but one of the leiomyomas with *FH* inactivation were solitary. Wild-type leiomyomas accounted for 29% of solitary leiomyomas and were seen with declining frequency in multiple leiomyomas. Due to the small number of samples, statistical testing was not possible for tumours other than *MED12*-positive tumours.

The majority of leiomyomas (350/361; 97%) were of conventional histology. Eleven were classified as histopathological variants, of which six were hypercellular, two showed bizarre nuclei, one was mitotically active, one was epithelioid, and one was a lipoleiomyoma. Two of the variant tumours displayed a *MED12* mutation, two showed HMGA2 overexpression, two indicated biallelic *FH* inactivation, and five were wild-type for all alterations studied. Detailed information on the variant leiomyomas is presented in Supplementary Table II.

To analyse associations between clinical variables and molecular alterations, the patients were divided into five groups based on the driver events in their leiomyomas (Fig. 2c). Group "*MED12*" includes patients whose every leiomyoma harboured a mutation in *MED12*, group "Multiple drivers" consists of patients with several leiomyomas with different drivers, and group "Wild-type" refers to the 47 patients (20%) whose leiomyomas were wild-type for all studied alterations. Table II presents the clinical variables analysed, divided by the driver groups as explained above. A statistical difference in driver distribution was present between patients of African descent and those with non-African background (p 0.016). Leiomyomas with FH deficiency were more common among patients

of African descent, while leiomyomas from non-African patients were more often wild-type for the studied alterations. However, multinomial logistic regression model did not yield a significant association between the groups. The median age at operation was 34 years. The distribution was significantly different between the driver groups (p 0.018), but in pairwise comparisons no statistical differences were seen. The number of leiomyomas removed varied significantly between the driver groups, and pairwise comparisons implied that the median number of leiomyomas removed in the *MED12* group was significantly higher than in the HMGA2 ($p < 0.001$), *FH* (p 0.012), and Wild-type ($p < 0.001$) groups. Likewise, the diameter of the largest leiomyoma was significantly different between the driver groups (p 0.007), and pairwise comparisons demonstrated a statistical difference between the *MED12* and HMGA2 groups (p 0.011), with a median diameter of 6.5 cm and 9 cm, respectively. Finally, a significant difference in the frequency of a subserosal location of leiomyoma emerged between the groups ($p < 0.001$), and it was further analysed by multinomial logistic regression. Compared with patients with only *MED12*-positive leiomyomas, patients in the other driver groups were less likely to have subserosal leiomyomas: OR for HMGA2 was 0.24 (0.099–0.56), OR for *FH* 0.18 (0.044–0.74), and OR for Wild-type 0.23 (0.11–0.47).

Since accumulation of 2SC is an indicator of non-functional FH, the *FH* coding region was sequenced in the 11 samples displaying positive 2SC immunohistochemical staining to identify the exact mutations. Heterozygotic mutations were found in eight samples. In five samples, the mutation was a missense change, one sample showed a nonsense mutation, one sample displayed a three-nucleotide deletion, and one sample had a single nucleotide deletion leading to a premature stop codon (Supplementary Table III). Normal tissue was available from five patients, and sequencing revealed a germline origin of the mutation in two of them.

Discussion

Here, we have analysed the molecular and clinical characteristics of leiomyomas obtained in a comprehensive, retrospective series of young leiomyoma patients. With a median age of 34 years, the patients were markedly younger than in earlier studies, which have mostly been conducted on hysterectomy patients. Our results indicate that the overall contribution of *MED12,* HMGA2, and *FH* alterations on leiomyomas from fertile-aged patients (83%) is comparable to those observed in perimenopausal women (80–90%) (Mehine et al., 2014). These three driver alterations are thus found in the great majority of all leiomyomas, irrespective of patients' age.

The most commonly affected gene was *MED12,* which was mutated in the great majority of tumours (71%). High *MED12* mutation frequency was specifically observed in multiple leiomyomas (over 80%), while only 43% of solitary leiomyomas displayed a mutation. The association of *MED12* mutations with multiple leiomyomas has also previously been described (Heinonen et al., 2014; McGuire et al., 2012), and in a Russian study population, the *MED12* mutation frequency was almost double (61%) in multiple leiomyomas compared to solitary tumours (32.5%) (Osinovskaya et al., 2016). In the present as well as in earlier studies (e.g. Mäkinen et al., 2011; Markowski et al., 2012; Heinonen et al., 2014), multiple *MED12* mutation-positive leiomyomas in a single uterus typically exhibited different mutations, suggesting independent clonal origin of the tumours. Our study also confirms the earlier observation that *MED12* mutation-positive leiomyomas are associated with a subserosal location and smaller tumour size (Heinonen et al., 2017).

HMGA2 overexpression was observed in 9% of leiomyomas, similar to frequencies reported earlier (Mehine et al., 2014; Bertsch et al., 2014). HMGA2-positive tumours presented mostly as solitary lesions, and these tumours were larger than those with a *MED12* mutation. These features have been associated with HMGA2 positivity also in previous studies (Markowski et al., 2014; Rein et al., 1998). A distinct molecular pathway has been suggested for leiomyomas displaying different driver

mutations, and at the DNA level these mutations have been mutually exclusive (Mehine et al., 2016). Two recent studies have, however, reported HMGA2 upregulation at the RNA level in the majority of leiomyomas, with some of the tumours harbouring also a *MED12* mutation (Galindo et al., 2018; Mello et al., 2018). Systematic analyses at DNA, RNA, and protein levels are now required to clarify whether the reported HMGA2 upregulation reflects a true mutational event that contributes to tumour development.

FH-deficient uterine leiomyomas are rare tumours, but they constitute a molecularly distinct and clinically important subset, especially when associated with HLRCC syndrome. Here, a positive staining in 2SC immunohistochemistry indicated FH-deficiency in 11 leiomyomas (3%). Ten of the 11 patients were operated on for a solitary tumour, and the median age of 32.5 years at operation did not differ from other driver groups. A personal or family history of cutaneous leiomyomas or renal cell carcinoma was not reported for any of the patients, but one patient had a previous diagnosis of HLRCC. Two tumours in the whole sample series were diagnosed with bizarre nuclei histology, and both of these were FH-deficient, supporting the previously observed association (Mäkinen et al., 2017; Zhang et al., 2018). Mutation screening revealed *FH* mutations in 8 out of 11 tumours. Four of the mutations have been reported earlier (Heikkinen et al., 2018; Kiuru et al., 2002; Bayley et al, 2008), and in silico predictions for the novel variants indicated three of them to be pathogenic (Kopanos et al., 2019). Limitations of the direct sequencing method in recognizing large deletions, insertions, or changes in the regulatory regions probably explain why a mutation was not identified in the remaining three samples. For the majority of patients with FH-deficient tumours, no archival normal tissue material was available, and a germline origin of the *FH* mutation could be confirmed in only two patients. Some FH-deficient tumours may thus be sporadic, even though somatic biallelic inactivation of *FH* is rare (Harrison et al., 2016, Lehtonen et al., 2004). In addition to the potential effect of recurring uterine leiomyomas on conceiving, identification of HLRCC patients is important

due to the increased risk for renal cancer. In the clinical setting, the diagnosis of leiomyoma with bizarre nuclei or personal or family history of uterine or cutaneous leiomyomas or renal cancer should arouse suspicion of HLRCC. If FH-deficient leiomyomas are seen, genetic counselling and mutation testing should be offered to the patient.

Altogether 11 tumours in the sample series (3%) were diagnosed as histopathological leiomyoma variants. This frequency is similar to that observed in tumours from Finnish hysterectomy patients (Heinonen et al., 2017). Six tumours harboured one of the three driver mutations supporting the previous observations that some other molecular alterations underlie a significant proportion of these tumours (Matsubara et al., 2013; Mäkinen et al., 2017). No occult leiomyosarcomas were observed among our study population. The age range (17–45 years) and a relatively small number of patients probably explain why there were no sarcomas (U.S. Food and Drug Administration 2017).

The proportion of patients suffering from infertility (24%) was notably higher than estimates for Finnish women based on self-reporting (16%) (Laatikainen et al., 2003). Although there is no evidence for a myomectomy improving fertility in patients with subserosal or intramural leiomyomas (Pritts et al., 2009), surgical treatment is perhaps offered more easily to all infertility patients with any leiomyoma.

Limitations

An obvious limitation of this study is that the leiomyoma samples have been collected for routine diagnostic purposes, not for research purposes. Therefore, this study only covers clinically significant tumours, while the smallest lesions might have been left in place during surgery. Moreover, especially in case of multiple leiomyomas, morcellation can make it difficult to distinguish all individual tumours. Dependence on diagnostic paraffin-embedded specimens poses challenges also in molecular

analyses due to DNA quality and possible loss of antigenicity in immunohistochemistry (Gaffney et al., 2018); this may have led to the underestimation of especially HMGA2-positive tumours. Hysteroscopic myomectomies have been omitted from this study because the FFPE tissue material is even more scarce in these samples. Evidently, the omission of hysteroscopic procedures has led to a limited number of submucosal leiomyomas (12/234 patients; 5%) in this study. On the other hand, the number of submucosal leiomyomas was very similar (44/763 tumours; 5.8%) in a study of tumours obtained by hysterectomy (Heinonen et al., 2017). For this reason, we believe that the lack of some submucosal leiomyomas has not caused a major bias in our study. Although the number of patients and samples included in the study is not small, the data are nevertheless partly descriptive due to the rarity of HMGA2-overexpressing and specifically FH-deficient leiomyomas. Larger sample series are needed to identify potentially statistically significant differences between different molecular and histological leiomyoma subtypes.

Interpretation and generalisability

Here, we have comprehensively analysed fertile-aged myomectomy patients, including both clinical analyses of patient data and molecular characterization of enucleated tumours. We show that the contribution of the three known driver alterations is comparable to tumours obtained from hysterectomies and that these mutations underlie the great majority of all leiomyomas, irrespective of patients' age. Although our study has focused on symptomatic leiomyomas, the distribution of *MED12*, HMGA2, and FH alterations is similar in hysterectomy studies that often include the smallest and clinically insignificant lesions. Additional studies in other ethnic groups, especially in women of African descent, are still warranted to validate this finding. *MED12* was the most commonly mutated gene and we confirm its' association with tumour size, multiple tumours, and subserosal location. Our findings imply that in solitary leiomyomas the distribution of genetic drivers differs from that in multiple leiomyomas: a notable portion of solitary lesions overexpressed HMGA2 and more than a

fourth of these tumours were wild-type for all studied alterations. Further studies are required to clarify the molecular background of leiomyomas not harbouring any of the established driver alterations.

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Authors' roles

AÄ, PP, PH, and PV contributed to the conception and design of the study. AÄ and TA collected the data and performed the experiments. PP and PH contributed to collecting the clinical data. AP performed the pathological analyses. AÄ, TH, and PV analysed the data. AÄ, TH, and PV wrote the manuscript. All authors commented on and approved the final version of the article.

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Conflict of interest

The authors declare no conflicts of interest.

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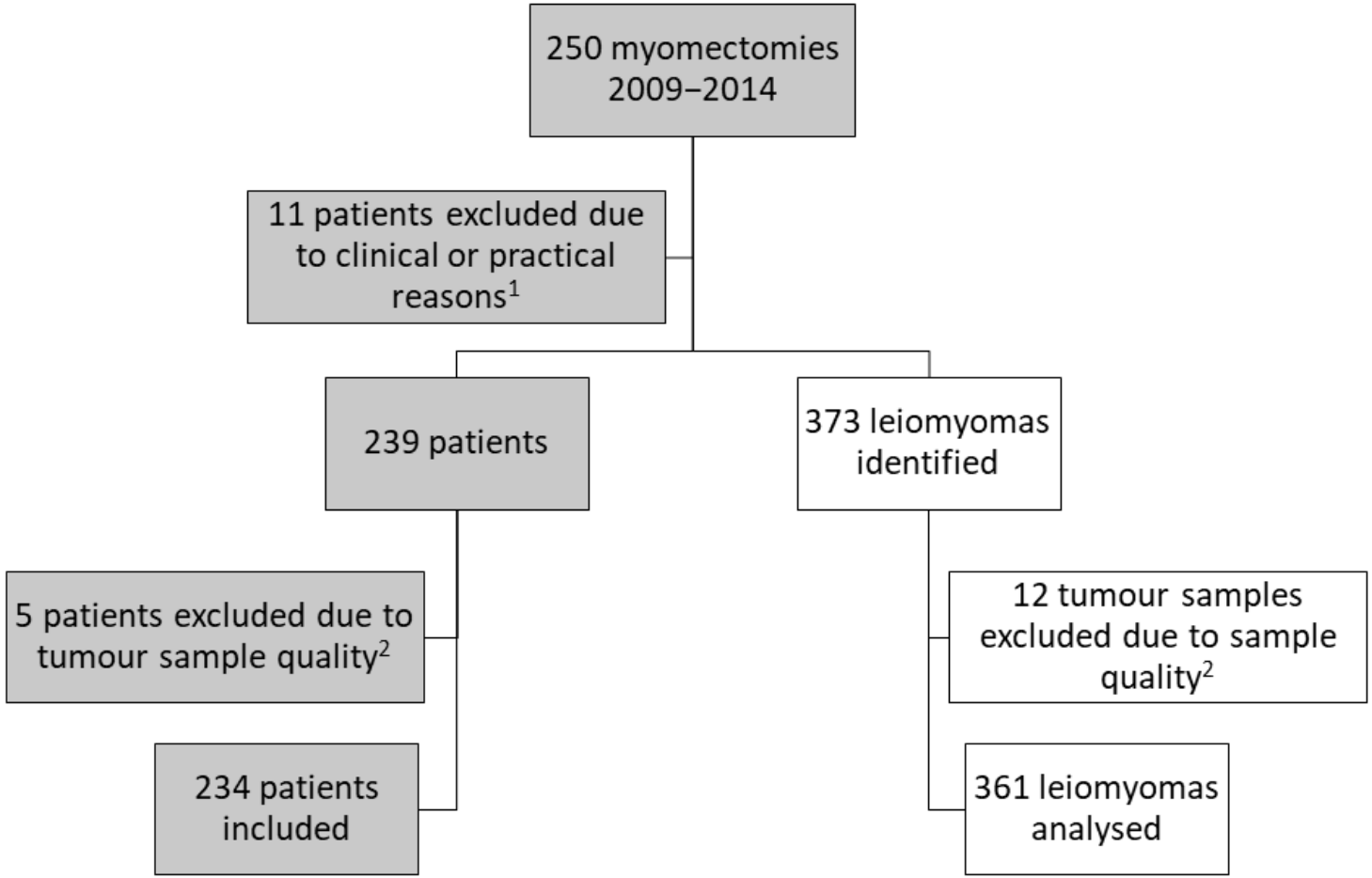
Figure Legends

Figure 1. Flow chart of the inclusion of myomectomy patients and uterine leiomyoma samples in the study. All tumour samples that could be identified as distinct leiomyomas by either molecular or clinical information were included in the study. Hysteroscopic myomectomies are not included in the study.

[1]Eleven patients were excluded due to clinical or practical reasons (two operations during the study period (n=1), missing samples or patient records (n=7), negative consent (n=1), postoperative

diagnosis other than leiomyoma (n=2)). [2]Twelve tumour samples were excluded due to poor sample quality or potential technical artefacts (necrotic sample material or low DNA quality (n=7), samples showing both mediator complex subunit 12 *(MED12)* mutations and high mobility group AT-hook 2 (HMGA2) positivity and subsequent inability to unambiguously determine whether these are true mutational events or technical artefacts (n=5)). Removal of these 12 tumours resulted in the exclusion of five patients who were operated on for a single leiomyoma.

Figure 2. Leiomyoma driver mutations in tumours obtained from myomectomies. (**A)** Frequencies of mediator complex subunit 12 *(MED12)* mutations, HMGA2 overexpression, FH deficiency, and wild-type (WT) tumours in 361 leiomyomas, and (**B**) in relation to the number of tumours removed from the same patient. Patients with 7, 10, 12, and 13 leiomyomas were not included as there was only one patient in each category. (**C)** Classification of 234 myomectomy patients based on which driver mutation was found in their leiomyoma. In each driver group, all tumours of the patient exhibited the same alteration, except for the “Multiple drivers” group, which includes patients with multiple leiomyomas with different drivers.


250 myomectomies 2009–2014
11 patients excluded due to clinical or practical reasons[1]
239 patients
373 leiomyomas identified
5 patients excluded due to tumour sample quality[2]
12 tumour samples excluded due to sample quality[2]
234 patients included
361 leiomyomas analysed

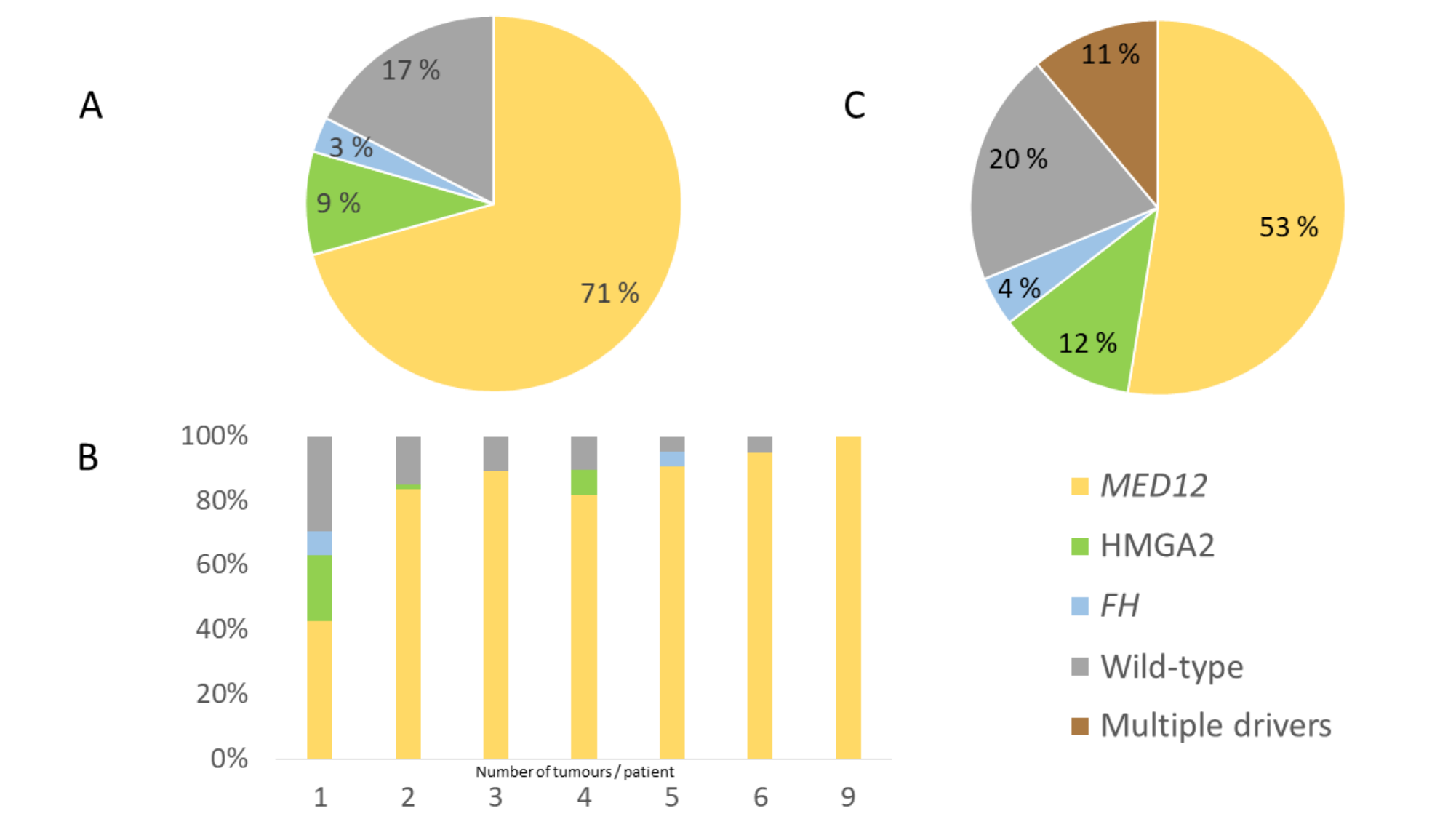

A
17 %
3 %
9 %
71 %
C
11 %
20 %
53 %
4 %
12 %
B
100%
80%
60%
40%
20%
0%
Number of tumours / patient
1
2
3
4
5
6
9
MED12
HMGA2
FH
Wild-type
Multiple drivers

Table I. Clinical characteristics of 234 fertile-aged myomectomy patients operated on at the Helsinki University Hospital in 2009–2014.

Patient characteristics (n= 234)	Number of patients (%)
Ethnicity	
Finnish*	177 (76)
African	21 (9)
Other[1]	34 (15)
Current smoker	41 (18)
Median BMI, kg/m2 (range)	23 (17–45)
Preoperative treatment with SPRM[2]	8 (3.4)
Preoperative treatment with GnRHa[3]	7 (3.0)
History of PID[4]	5 (2.1)
Diagnosis of endometriosis	33 (14)
Prior myomectomy	15 (6.4)
Median age at menarche, years (range)	13 (9–17)
Number of prior pregnancies	
0	153 (65)
1	41 (18)
2	22 (9)
3–9	18 (8)
Infertility	57 (24)
Median age at operation, years (range)	34 (17–45)
Surgical method	
Abdominal surgery	119 (51)
Laparoscopy	97 (41)
Robotic assisted laparoscopy	18 (8)
Morcellator used	94 (40)

* Finnish are white Caucasians by ethnicity, but often analysed as a separate group due to unique genetic background.

[1] European other than Finnish, Asian, Latin American

[2] Selective progesterone receptor modulator

[3] Gonadotrophin-releasing hormone agonist

[4] Pelvic inflammatory disease

Table II. Clinical characteristics of 234 myomectomy patients divided by driver alterations in their leiomyomas.

Characteristics	*MED12*[1]	HMGA2[2]	FH[3]	Wild-type	Multiple drivers	p
Number of patients, n=234	123 (53)	28 (12)	10 (4.3)	47 (20)	26 (11)	
Ethnicity						0.016*
Finnish[4] and other non-African[5], n=211	111 (53)	26 (12)	7 (3.3)	46 (22)	21 (10)	
African, n=21	11 (52)	1 (4.8)	3 (14)	1 (4.8)	5 (24)	
Median age at operation, years (range)	35 (21–44)	32 (23–43)	32.5 (24–39)	32 (17–45)	35 (27–44)	0.018*
Median body mass index, kg/m2 (range)	23.5 (17–45)	23.5 (18–31)	23 (18–31)	23 (18–41)	24 (19–38)	0.648
Median age at menarche, years (range)	13 (9–16)	12 (11–15)	14 (10–16)	13 (10–17)	13 (11–16)	0.544
Use of hormonal contraception	29 (24)	6 (21)	4 (40)	12 (26)	4 (15)	0.627
Endometriosis diagnosed	22 (18)	1 (3.6)	1 (10)	7 (15)	2 (7.7)	0.299
Prior myomectomy	6 (4.9)	2 (7.1)	2 (20)	1 (2.1)	4 (15)	0.057
Infertility	36 (29)	3 (11)	2 (20)	9 (19)	7 (27)	0.254
Median preoperative number of pregnancies (range)	0 (0–6)	0 (0–4)	1 (0–3)	0 (0–9)	0 (0–2)	0.088
Median number of leiomyomas removed (range)	2 (1–12)	1 (1–4)	1 (1)	1 (1–4)	3 (1–13)	<0.001*
Median diameter of the largest leiomyoma (range)	6.5 (1.5–17.5)	9 (4.5–20)	6 (3–12)	8 (3–20)	8 (2–14)	0.007*
Leiomyoma classification						
Submucosal	6 (4.9)	0	1 (10)	3 (6.4)	2 (7.7)	0.461
Intramural	65 (53)	17 (61)	6 (60)	32 (68)	15 (58)	0.479
Subserosal	85 (69)	10 (36)	3 (30)	16 (34)	19 (73)	<0.001*

Values are number and percentage unless otherwise indicated. Percentages within driver groups are shown, except for Number of patients and Ethnicity.

[1] Mediator complex subunit 12; [2] High mobility group AT-hook 2; [3] Fumarate hydratase

[4] Finnish are white Caucasians by ethnicity, but often analysed as a separate group due to unique genetic background.

[5] European other than Finnish, Asian, and Latin American

*p<0.05 is considered statistically significant

Supplementary Table I. Molecular characteristics and histopathology of 361 uterine leiomyomas from 234 patients.

Patient ID	Removed leiomyomas (n)	Identified leiomyomas (n)	Histopathology	*MED12*[1]	2-SC[2]	HMGA2[3]
1	1	1		–	positive	–
2	1	1		c.131G>A;p.G44D	–	–
3	1	1		–	–	–
4	1	1		–	positive	–
5	1	1		c.130G>A;p.G44S	–	–
6	1	1		–	–	positive
7	1	1	Epithelioid	–	–	–
8	1	1		–	–	positive
9	1	1		–	–	–
10	1	1		–	–	–
11	1	1	Mitotically active	–	–	positive
12	1	1		–	–	–
13	1	1		–	–	positive
14	1	1		–	positive	–
15	1	1		–	–	–
16	1	1		–	–	positive
17	1	1		c.131G>A;p.G44D	–	–
18	1	1		c.131G>A;p.G44D	–	–
19	1	1		–	positive	–
20	1	1		c.105_137del33;p.E35_N46delinsD	–	–
21	1	1		c.131G>A;p.G44D	–	–
22	1	1		c.100-1_136del38;p.D34_N46del, possible splice effect	–	–
23	1	1		–	–	–
24	1	1		–	–	–
25	1	1	Bizarre nuclei	c.46C>A;p.R16R[4]	positive	–

26	1	1		c.131G>T;p.G44V	–	–
27	1	1		c.131G>A;p.G44D	–	–
28	1	1		–	–	–
29	1	1		–	positive	–
30	1	1		–	–	positive
31	1	1		–	–	positive
32	1	1		c.131G>T;p.G44V	–	–
33	1	1		c.131G>C;p.G44A	–	–
34	1	1	Bizarre nuclei	–	positive	–
35	1	1		–	–	–
36	1	1		–	–	positive
37	1	1		c.131G>T;p.G44V	–	–
38	1	1		c.130G>A;p.G44S	–	–
39	1	1		c.130G>A;p.G44S	–	–
40	1	1		–	–	positive
41	1	1		c.131G>T;p.G44V	–	–
42	1	1		–	–	–
43	1	1		c.129_146del18;p.Q43_P49delinsH	–	–
44	1	1		c.130G>T;p.G44C	–	–
45	1	1		c.108_109insCAGGATGAACTG;p.L36_T37insQDEL	–	–
46	1	1		c.131G>A;p.G44D	–	–
47	1	1		–	–	positive
48	1	1		–	–	–
49	1	1		c.102_140del39;p.E35_N47del	–	–
50	1	1		–	–	–
51	1	1	Hypercellular	–	–	–
52	1	1		c.131G>T;p.G44V	–	–
53	1	1		c.131G>A;p.G44D	–	–
54	1	1		–	–	–
55	1	1		c.130G>A;p.G44S	–	–
56	1	1		–	–	–

57	1	1	c.139_159del21;p.N47_G53del	–	–
58	1	1	–	–	–
59	1	1	–	–	–
60	1	1	–	–	positive
61	1	1	–	–	positive
62	1	1	–	–	–
63	1	1	–	–	–
64	1	1	c.131G>A;p.G44D	–	–
65	1	1	–	–	positive
66	1	1	–	–	positive
67	1	1	–	–	–
68	1	1	c.100-8T>A;p.E33_D34insPQ	–	–
69	1	1	–	–	–
70	1	1	c.131G>A;p.G44D	–	–
71	1	1	–	–	positive
72	1	1	–	–	positive
73	1	1	c.131G>T;p.G44V	–	–
74	1	1	–	–	–
75	1	1	–	positive	–
76	1	1	c.100-8T>A;p.E33_D34insPQ	–	–
77	1	1	c.131G>A;p.G44D	–	–
78	1	1	c.132_150del19insG;p.F45_A50del	–	–
79	1	1	–	–	positive
80	1	1	–	–	–
81	1	1	–	positive	–
82	1	1	–	–	–
83	1	1	–	positive	–
84	1	1	c.130G>C;p.G44R	–	–
85	1	1	–	–	positive
86	1	1	–	–	–
87	1	1	–	–	–

88	1	1		–	–	positive
89	1	1		c.131G>T;p.G44V	–	–
90	1	1		c.131G>A;p.G44D	–	–
91	1	1		–	–	positive
92	1	1		–	–	positive
93	1	1		c.100-17_104del22;p.D34_E35, possible splice effect	–	–
94	1	1		–	–	positive
95	1	1		–	–	positive
96	1	1	Hypercellular	–	–	–
97	1	1		c.131G>A;p.G44D	–	–
98	1	1		–	–	–
99	1	1		–	–	positive
100	1	1		c.138_158del21;p.N46_G53delinsK	–	–
101	1	1		c.124_135del12p;K42_F45del	–	–
102	1	1		–	–	positive
103	1	1		–	–	positive
104	1	1		c.131G>T;p.G44V	–	–
105	1	1		–	–	positive
106	1	1		–	–	–
107	1	1		c.131G>A;p.G44D	–	–
108	1	1		–	–	–
109	1	1		c.131G>A;p.G44D	–	–
110	1	1		c.130G>C;p.G44R	–	–
111	1	1		–	–	–
112	1	1		c.131G>A;p.G44D	–	–
113	1	1		–	–	positive
114	1	1		c.130G>C;p.G44R	–	–
115	1	1		–	–	–
116	1	1		c.131G>T;p.G44V	–	–
117	1	1		–	–	–
118	1	1	Hypercellular	c.131G>A;p.G44D	–	–

119	1	1	Hypercellular	–	–	–
120	1	1		c.121_132del12;p.V41_G44del	–	–
121	1	1		c.130_131del2insAA;p.G44N	–	–
122	1	1		c.131G>T;p.G44V	–	–
123	1	1		c.131G>A;p.G44D	–	–
124	1	1		c.131G>T;p.G44V	–	–
125	1	1		c.119_148del30;p.N40_A50delinsT	–	–
126	1	1		–	–	–
127	1	1		c.130G>T, c.131G>T;p.G44F	–	–
128	1	1		c.130G>A;p.G44S	–	–
129	1	1		–	–	–
130	1	1		–	–	–
131	1	1		–	–	–
132	1	1		c.107T>G;p.L36R	–	–
133	1	1		–	–	–
134	1	1		c.131G>A;p.G44D	–	–
135	1	1		c.130G>T;p.G44C	–	–
136	1	1		c.131G>C;p.G44A	–	–
137	2	1		c.130G>T;p.G44C	–	–
143	2	1		c.131G>C;p.G44A	–	–
145	2	2		c.130G>A;p.G44S	–	–
145	2	2		c.130G>T;p.G44C	–	–
148	2	2		c.84_98del15;p.D28_K32del	–	–
148	2	2		c.130G>A;p.G44S	–	–
149	2	2		–	–	–
149	2	2		c.131G>A;p.G44D	–	–
152	2	2		–	–	–
152	2	2		c.131G>T;p.G44V	–	–
153	2	1		c.107_142del36;p.L36_N47del	–	–
157	2	2		c.131G>A;p.G44D	–	–
157	2	2		c.130G>A;p.G44S	–	–

158	2	2	–	–	–
158	2	2	c.131G>A;p.G44D	–	–
160	2	2	c.100-8T>A;p.E33_D34insPQ	–	–
160	2	2	c.107T>G;p.L36R	–	–
166	2	1	c.107T>G;p.L36R	–	–
169	2	1	–	–	–
170	2	2	c.131G>T;p.G44V	–	–
170	2	2	c.131G>C;p.G44A	–	–
171	2	2	–	–	–
171	2	2	–	–	–
172	2	2	c.130G>C;p.G44R	–	–
172	2	2	c.131G>T;p.G44V	–	–
176	2	1	–	–	–
178	2	1	c.130G>A;p.G44S	–	–
179	2	2	c.131G>A;p.G44D	–	–
179	2	2	c.131G>A;p.G44D	–	–
180	2	2	c.107T>G;p.L36R	–	–
180	2	2	c.131G>C;p.G44A	–	–
182	2	2	c.122_148del27;p.V41_P49del	–	–
182	2	2	c.131G>A;p.G44D	–	–
184	2	1	c.100-10_129del40;p.D34_Q43del, possible splice effect	–	–
185	2	2	c.119_145del27;p.N40_P49delinsT	–	–
185	2	2	c.130G>C;p.G44R	–	–
186	2	1	c.131G>A;p.G44D	–	–
187	2	2	c.130G>A;p.G44S	–	–
187	2	2	c.107T>G;p.L36R	–	–
190	2	1	c.130G>A;p.G44S	–	–
195	2	2	c.121_144del24;p.V41_Q48del	–	–
195	2	2	c.130G>A;p.G44S	–	–
196	2	2	c.131G>A;p.G44D	–	–

196	2	2	c.121_144del24;p.V41_Q48del	–	–
200	2	2	c.130G>A;p.G44S	–	–
200	2	2	c.131G>A;p.G44D	–	–
201	2	2	c.130G>A;p.G44S	–	–
201	2	2	c.131G>A;p.G44D	–	–
206	2	2	–	–	positive
206	2	2	c.110_118del9 & c.122T>G;p.T37_ L39del & p.V41G	–	–
207	2	1	c.130G>A;p.G44S	–	–
208	2	1	c.131G>A;p.G44D	–	–
212	2	1	c.39C>T;p.P13P[1], c.54G>A;p.R18R[4]	–	–
218	2	1	c.131G>A;p.G44D	–	–
223	2	1	c.124_144del21;p.K42_Q48del	–	–
224	2	1	c.131G>A;p.G44D	–	–
225	2	2	c.130G>A;p.G44S	–	–
225	2	2	c.107T>G;p.L36R	–	–
227	2	2	–	–	–
227	2	2	c.130G>A;p.G44S	–	–
230	2	2	c.100-8T>A;p.E33_D34insPQ	–	–
230	2	2	c.128A>C;p.Q43P	–	–
231	2	2	–	–	–
231	2	2	c.131G>T;p.G44V	–	–
232	2	1	c.130G>A;p.G44S	–	–
233	2	2	c.131G>T;p.G44V	–	–
233	2	2	c.131G>A;p.G44D	–	–
138	3	3	c.127_138del;p.Q43_N46del	–	–
138	3	3	c.131G>C;p.G44A	–	–
138	3	3	c.107T>G;p.L36R	–	–
142	3	2	c.100_117del18;p.D34_L39del	–	–
142	3	2	c.130G>A;p.G44S	–	–
150	3	3	c.131G>A;p.G44D	–	–
150	3	3	c.131G>T;p.G44V	–	–

150	3	3	c.122_148del27;p.V41_P49del	–	–
159	3	3	c.100-6_129del36;p.D34_Q43del	–	–
159	3	3	c.131G>T;p.G44V	–	–
159	3	3	c.131G>A;p.G44D	–	–
162	3	2	c.100-8T>A;p.E33_D34insPQ	–	–
162	3	2	c.130G>C;p.G44R	–	–
163	3	4[5]	c.131G>T;p.G44V	–	–
163	3	4[5]	c.131G>A;p.G44D	–	–
163	3	4[5]	c.130G>A;p.G44S	–	–
163	3	4[5]	c.100-8T>A;p.E33_D34insPQ	–	–
164	3	3	c.131G>A;p.G44D	–	–
164	3	3	c.131G>A;p.G44D	–	–
164	3	3	c.115_135del21;p.L39_F45del	–	–
167	3	1	c.100-6_129del36;p.D34_Q43del, possible splice effect	–	–
181	3	3	c.145_162del18;p.P49_D54del	–	–
181	3	3	c.130G>A;p.G44S	–	–
181	3	3	c.124_147del24;p.K42_P49del	–	–
183	3	2	c.130G>A;p.G44S	–	–
183	3	2	c.130G>C;p.G44R	–	–
193	3	3	–	–	–
193	3	3	c.131G>A;p.G44D	–	–
193	3	3	c.130G>A;p.G44S	–	–
199	3	2	c.130G>A;p.G44S	–	–
199	3	2	c.131G>A;p.G44D	–	–
210	3	1	c.131G>T;p.G44V	–	–
211	3	2	–	–	–
211	3	2	–	–	–
215	3	1	c.121_129del9insTTG;p.V41_Q43delinsL	–	–
216	3	3	c.131_139del9;p.G44_N46del, N47D	–	–
216	3	3	c.130G>A;p.G44S	–	–

216	3	3		c.130G>A;p.G44S	–	–
222	3	3		c.84_98del15;p.D28_K32del	–	–
222	3	3		c.102_128del27;p.D34E,p.E35_Q43del	–	–
222	3	3		c.131G>T;p.G44V	–	–
228	3	3		–	–	–
228	3	3		c.100-8_129del38;p.D34_Q43del, possible splice effect	–	–
228	3	3		c.100-8T>A;p.E33_D34insPQ	–	–
234	3	3		c.15G>A;p.G5G[4]	–	–
234	3	3		c.130G>C;p.G44R	–	–
234	3	3		c.116_154del39;p.L39_V51del	–	–
194	4	4		c.130G>T;p.G44C	–	–
194	4	4		c.131G>A;p.G44D	–	–
194	4	4		c.133_144del12;p.F45_Q48del	–	–
194	4	4		c.133_144del12;p.F45_Q48del	–	–
144	4	4		c.130G>A;p.G44S	–	–
144	4	4		c.130G>A;p.G44S	–	–
144	4	4		c.130G>A;p.G44S	–	–
144	4	4		c. 130G>A, 131G>T;p.G44I	–	–
151	4	2		–	–	–
151	4	2		–	–	–
156	4	3		c.133_147del15;p.F45_P49del	–	–
156	4	3		c.120_140del21insAAC;p.V41_N47del	–	–
156	4	3		c.130G>A;p.G44S	–	–
161	4	2		c.82_99del18;p.D28_E33del	–	–
161	4	2		c.131G>A;p.G44D	–	–
165	4	2		c.117_128del12;p.N40_Q43delinsL	–	–
165	4	2		–	–	positive
173	4	1		–	–	positive
174	4	4	Lipoleiomyoma	–	–	positive
174	4	4		c.131G>A;p.G44D	–	–

174	4	4		c.130G>T;p.G44C	–	–
174	4	4		c.130G>C;p.G44R	–	–
175	4	1	Hypercellular	c.131G>C;p.G44A	–	–
177	4	4		c.130G>T;p.G44C	–	–
177	4	4		c.130G>C;p.G44R	–	–
177	4	4		c.130G>A;p.G44S	–	–
177	4	4		c.130G>C;p.G44R	–	–
191	4	3		c.131G>C;p.G44A	–	–
191	4	3		c.130G>A;p.G44S	–	–
191	4	3		c.129_143del15;p.Q43_N47del	–	–
197	4	4		–	–	–
197	4	4		c.130G>T;p.G44C	–	–
197	4	4		c.131G>A;p.G44D	–	–
197	4	4		c.131G>C;p.G44A	–	–
205	4	1		c.131G>A;p.G44D	–	–
214	4	1		–	–	–
219	4	1		c.146_c.166del21;p.P49_E55del	–	–
221	4	2		c.131G>A;p.G44D	–	–
221	4	2		c.131G>C;p.G44A	–	–
139	5	5		c.131G>T;p.G44V	–	–
139	5	5		c.100-2_129del32;p.D34_Q43del	–	–
139	5	5		c.130G>T;p.G44C	–	–
139	5	5		c.130G>A;p.G44S	–	–
139	5	5		c.131G>T;p.G44V	–	–
146	5	2		c.131G>A;p.G44D	–	–
146	5	2		c.130G>T;p.G44C	–	–
188	5	3		c.126_140del15;p.K42_N46del	–	–
188	5	3		c.123_152del30;p.K42_V51del	–	–
188	5	3		c.123_152del30;p.K42_V51del	–	–
189	5	1		c.131G>A;p.G44D	–	–
192	5	4		c.131G>A;p.G44D	–	–

192	5	4	c. 128A>C;p.Q43P	–	–
192	5	4	c.130G>T;p.G44C	–	–
192	5	4	c.100-10_135del46;p.D34_F45del, possible splice effect	–	–
217	5	3	c.131G>A;p.G44D	–	–
217	5	3	c.130G>A;p.G44S	–	–
217	5	3	–	positive	–
220	5	4	–	–	–
220	5	4	c.131G>A;p.G44D	–	–
220	5	4	c.130G>C;p.G44R	–	–
220	5	4	c.101_112del12;p.D34_T37del	–	–
141	6	2	–	–	–
141	6	2	c.133_147del15;p.F45_P49del	–	–
154	6	2	c.130G>C;p.G44R	–	–
154	6	2	c.131G>A;p.G44D	–	–
168	6	5	c.131G>A;p.G44D	–	–
168	6	5	c.100-8T>A;p.E33_D34insPQ	–	–
168	6	5	c.131G>A;p.G44D	–	–
168	6	5	c.130G>A;p.G44S	–	–
168	6	5	c.130G>C;p.G44R	–	–
202	6	2	c.131G>C;p.G44A	–	–
202	6	2	c.122T>A;p.V41E	–	–
209	6	3	c.130G>A;p.G44S	–	–
209	6	3	c.131G>A;p.G44D	–	–
209	6	3	c.131G>A;p.G44D	–	–
213	6	1	c.131G>A;p.G44D	–	–
229	6	5	c.130G>A;p.G44S	–	–
229	6	5	c.131G>A;p.G44D	–	–
229	6	5	c.130G>T;p.G44C	–	–
229	6	5	c.131G>T;p.G44V	–	–
229	6	5	c.130G>A;p.G44S	–	–

147	7	4	Hypercellular	–	–	–
147	7	4		c.131G>C;p.G44A	–	–
147	7	4		c.131G>A;p.G44D	–	–
147	7	4		c.131G>T;p.G44V	–	–
198	9	3		c.131G>A;p.G44D	–	–
198	9	3		c.131G>A;p.G44D	–	–
198	9	3		c.130G>A;p.G44S	–	–
203	9	6		c.130G>A;p.G44S	–	–
203	9	6		c.100-8T>A;p.E33_D34insPQ	–	–
203	9	6		c.107T>G;p.L36R	–	–
203	9	6		c.131G>A;p.G44D	–	–
203	9	6		c.131G>C;p.G44A	–	–
203	9	6		c.131G>T;p.G44V	–	–
226	9	2		c.130G>T;p.G44C	–	–
226	9	2		c.131G>T;p.G44V	–	–
140	10	2		c.130G>A;p.G44S	–	–
140	10	2		c.124_144del21;p.K42_Q48del	–	–
155	12	8		c.100-8T>A;p.E33_D34insPQ	–	–
155	12	8		c.131G>A;p.G44D	–	–
155	12	8		c.130G>T;p.G44C	–	–
155	12	8		c.78_95del18;p.Q27_K32del	–	–
155	12	8		c.133_147del15;p.F45_P49del	–	–
155	12	8		c.123_134del12;p.K42_F45del	–	–
155	12	8		c.126_137del12;p.K42_F45del	–	–
155	12	8		c.131G>C;p.G44A	–	–
204	13	5		–	–	–
204	13	5		c.131G>A;p.G44D	–	–
204	13	5		c.133_147del15;p.F45_P49del	–	–
204	13	5		c.118_159del42;p.N40_G53del	–	–
204	13	5		c.131G>C;p.G44A	–	–

[1] Mediator complex subunit 12
[2] 2-succinylcysteine, indirect method for detecting fumarate hydratase -deficiency
[3] High mobility group AT-hook 2
[4] Synonymous variant, not considered as a mutation in the statistical analyses
[5] According to medical records, 3 leiomyomas were removed, but 4 different *MED12* mutations were identified from the tissue material

Supplementary Table II. Mutation status and selected clinical information on 11 histopathological variant leiomyomas.

Patient	Removed leiomyomas (n)	Histopathology	*MED12*[1] mutation	HMGA2[2]	2SC[3]	Age (years)	Ethnicity[4]	Leiomyoma diameter (cm)	Leiomyoma classification
7	1	Epithelioid	-	-	-	26	Finnish	10	Subserosal
11	1	Mitotically active	-	positive	-	28	Finnish	9	Intramural
25	1	Bizarre nuclei	-	-	positive	24	African	9	Subserosal
34	1	Bizarre nuclei	-	-	positive	27	Finnish	5	Subserosal
51	1	Hypercellular	-	-	-	30	Asian	6	Intramural
96	1	Hypercellular	-	-	-	29	Finnish	4	Intramural
118	1	Hypercellular	c.131G>A;p.G44D	-	-	33	Finnish	6,5	Subserosal
119	1	Hypercellular	-	-	-	34	Finnish	7	Subserosal
174	4	Lipoleiomyoma	-	positive	-	40	Finnish	na	Subserosal
175	4	Hypercellular	c.131G>C;p.G44A	-	-	37	Finnish	na	Subserosal
147	7	Hypercellular	-	-	-	33	Finnish	na	na

[1] Mediator complex subunit 12

[2] High mobility group AT-hook 2

[3] 2-succinylcysteine, indirect method for detecting fumarate hydratase -deficiency

[4] Finnish are white Caucasians by ethnicity, but often analysed as a separate group due to unique genetic background.

Supplementary Table III. Mutation status of 11 fumarate hydratase -deficient uterine leiomyomas.

Patient ID	*FH** Mutation	In Silico Prediction (Varsome)	Reported	Germline
1	c.1043G>T;p.G348V	Likely pathogenic	No	Normal tissue samples not available
4	Not found			
14	c.1481_1483delCAG;p.A494del	Likely pathogenic	No	No
19	c.151C>T;p.R51W	Likely pathogenic	Kiuru et al 2002	Normal tissue samples not available
25	Not found			
29	Not found			
34	c.1027C>T;p.R343STOP	Pathogenic	Bayley et al 2008	Normal tissue samples not available
75	c.911delC;p.P304fs	Pathogenic	Heikkinen et al 2018	Yes
81	c.1343T>C;p.L448P	Likely pathogenic	No	No
83	c.1256C>T;p.S419L	Likely pathogenic	No	Yes
217	c.152G>T;p.R51L	Likely pathogenic	Kiuru et al 2002	No

*Fumarate hydratase