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MED12 mutations and *FH* inactivation are mutually exclusive in uterine leiomyomas

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Background: Uterine leiomyomas from hereditary leiomyomatosis and renal cell cancer (HLRCC) patients are driven by fumarate hydratase (*FH*) inactivation or occasionally by mediator complex subunit 12 (*MED12*) mutations. The aim of this study was to analyse whether *MED12* mutations and *FH* inactivation are mutually exclusive and to determine the contribution of *MED12* mutations on HLRCC patients' myomagenesis.

Methods: *MED12* exons 1 and 2 mutation screening and 2SC immunohistochemistry indicative for FH deficiency was performed on a comprehensive series of HLRCC patients' (122 specimens) and sporadic (66 specimens) tumours. Gene expression analysis was performed using Affymetrix GeneChip Human Exon Arrays (Affymetrix, Santa Clara, CA, USA).

Results: Nine tumours from HLRCC patients harboured a somatic *MED12* mutation and were negative for 2SC immunohistochemistry. All remaining successfully analysed lesions (107/116) were deficient for FH. Of sporadic tumours, 35/64 were *MED12* mutation positive and none displayed a FH defect. In global gene expression analysis FH-deficient tumours clustered together, whereas HLRCC patients' *MED12* mutation-positive tumours clustered together with sporadic *MED12* mutation-positive tumours.

Conclusions: Somatic *MED12* mutations and biallelic *FH* inactivation are mutually exclusive in both HLRCC syndrome-associated and sporadic uterine leiomyomas. The great majority of HLRCC patients' uterine leiomyomas are caused by *FH* inactivation, but incidental tumours driven by somatic *MED12* mutations also occur. These *MED12* mutation-positive tumours display similar expressional profiles with their sporadic counterparts and are clearly separate from FH-deficient tumours.

Uterine leiomyomas, or fibroids, are very common benign tumours among reproductive-aged women; nearly 70% are affected by these lesions before age 50 (Day Baird *et al*, 2003). Approximately one quarter of the patients suffer from symptoms such as abdominal pain, abnormal uterine bleeding, subfertility, and complications during pregnancy (Stewart, 2001). Uterine leiomyomas originate

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from the smooth muscle layer of the uterus and most are formed sporadically. Several independent studies on various populations have shown that ~70% of uterine leiomyomas harbour specific mutations in mediator complex subunit 12 (*MED12*) (Mäkinen *et al*, 2011; Je *et al*, 2012; McGuire *et al*, 2012). All observed changes have been missense mutations or small in-frame insertions and deletions in exons 1 and 2 affecting a highly conserved area of the gene (Mäkinen *et al*, 2011; Je *et al*, 2012; McGuire *et al*, 2012; Kämpjärvi *et al*, 2014). Other recurrently, albeit less frequently, observed alterations include genomic rearrangements affecting *HMGA2* in chromosome 12q15 and *RAD51B* in 14q23-24 (Ingraham *et al*, 1999; Sandberg, 2005). Recurrent non-random translocations and deletions have also been observed in other chromosomal areas, such as *COL4A5* and *COL4A6* locus in X chromosome, 6p21 (*HMGA1*), and 7q22 (Van de Ven, 1998; Sandberg, 2005; Mehine *et al*, 2013).

In addition to sporadic lesions, uterine leiomyomas are a feature of hereditary leiomyomatosis and renal cell cancer syndrome (HLRCC, OMIM #150800). HLRCC is a tumour predisposition syndrome caused by heterozygous germ-line mutations in fumarate hydratase (*FH*; Launonen *et al*, 2001; Tomlinson *et al*, 2002). The gene encodes fumarase enzyme, which catalyses the hydration of fumarate to L-malate in tricarboxylic acid cycle. *FH* is a tumour suppressor, and syndrome-associated lesions display biallelic *FH* inactivation through loss of heterozygosity (LOH) or inactivating mutation in the wild-type allele. In addition to uterine leiomyomas, *FH* mutations predispose individuals to cutaneous leiomyomas, and more rarely, to aggressive renal cell cancer.

Based on our recent preliminary results, somatic *MED12* mutations and biallelic *FH* inactivation might exclude each other as drivers of leiomyomagenesis (Mäkinen *et al*, 2013; Mehine *et al*, 2013; Mäkinen *et al*, 2014). Here, our aim was to systematically analyse whether these phenomena are truly mutually exclusive and to clarify the role of *MED12* in the tumourigenesis of HLRCC patients' uterine leiomyomas. We collected a comprehensive series of HLRCC patients' samples totalling 122 uterine leiomyomas from 27 individuals. Sixty-six sporadic conventional uterine leiomyomas were also included in the study. *MED12* mutation status was analysed by direct sequencing and biallelic *FH* inactivation was determined by 2SC immunohistochemistry, which has proven to be an accurate and unambiguous method to detect FH deficiency (Bardella *et al*, 2011). The effects of *MED12* and *FH* alterations on global expression profiles and clustering of the tumours were also studied.

MATERIALS AND METHODS

Subjects and ethical approval of the study. Twenty-seven Finnish HLRCC patients, representing 11 families, and 66 individuals with sporadic leiomyomas were included in the study. Altogether 188 uterine leiomyoma samples were collected, 122 tumours from HLRCC patients (89 formalin-fixed paraffin-embedded (FFPE) and 33 fresh frozen samples) and 66 tumours (FFPE samples) from individuals with sporadic uterine leiomyomas. Samples were collected either after signed informed consent or after the approval by the National Supervisory Authority for Welfare and Health. Sporadic samples were collected and anonymised after the approval by the director of the health care unit. The study was approved by the Ethics Review Board of the Hospital District of Helsinki and Uusimaa (HUS), Helsinki, Finland.

DNA/RNA extraction and sequencing. Genomic DNA was extracted from the FFPE samples with a standard phenol–chloroform extraction method or with NucleoSpin DNA FFPE XS Kit (Macherey-Nagel GmbH & Co KG, Düren, Germany), and from fresh frozen tissue samples with FastDNA Kit (MP Biomedicals LLC, Solon, OH, USA). Total RNA was extracted with TRI Reagent (Molecular Research Center Incorporated, Cincinnati, OH, USA). *MED12* and *FH* mutation screenings were performed by direct sequencing. Sanger sequencing was carried out at the Institute of Molecular Medicine Finland, Technology Center, Helsinki, Finland, using an Applied Biosystems ABI3730 Automatic DNA Sequencer (Life technologies, Thermo Fisher Scientific, Waltham, MA, USA). Sequences were analysed both manually and with the Mutation Surveyor software (Softgenetics, State College, PA, USA). *MED12* NM_005120.2 and *FH* NM_000143.3 were used as reference sequences. Primers used in the sequencing are listed in the Supplementary Table 1.

Loss of heterozygosity analysis. LOH at the *FH* locus was assessed from fresh frozen tumour samples harbouring both a germ-line *FH* mutation and a somatic *MED12* mutation. LOH was analysed by sequencing five independent PCR reactions and comparing the heights of wild-type and mutant peaks in the chromatograms. LOH was scored when the height of wild-type allele peak was repeatedly lower than the mutant allele peak.

Tissue microarray construction. To determine the most suitable areas of the samples for tissue-microarray (TMA), haematoxylin and eosin staining was performed to sections cut from all FFPE samples. Tissue samples were analysed and representative tumour areas were marked by a gynaecological pathologist (RB). Four 0.8-mm tumour cores were punched from the original sample block with a manual tissue arrayer (MTA-I, Beecher Instruments, Sun Prairie, WI, USA) and inserted in TMA paraffin block. Normal myometrium samples were included in the TMA as internal controls.

Immunohistochemistry. Biallellic inactivation of *FH* was assessed with 2SC immunohistochemical staining performed with EnVision + kit (Dako, Carpinteria, CA, USA). Staining is based on the recognition of S-(2-succinyl) cysteine modified (succinated) proteins which are formed in FH deficient cells as a result of the accumulation of fumarate (Nagai *et al*, 2007; Bardella *et al*, 2011). Tissue sections (5 μm) were incubated with the anti-2SC antibody (1 : 2000) at +4 °C, o/n (FFPE samples) or at room temperature for an hour (fresh frozen samples). Antibody binding was detected with anti-rabbit horseradish peroxidase polymer. Stainings were assessed by a pathologist (RB) and the sample was scored as positive (+) when the great majority of cells displayed both nuclear and cytoplasmic staining indicating accumulation of fumarate and succinated proteins and thus biallelic inactivation of *FH*. Samples displaying no staining or rare samples showing low cytoplasmic positivity in single cells were scored as negative (−), indicating the cells to retain sufficient FH activity.

Gene expression profiling and hierarchical clustering analysis. Gene expression profiles of 14 leiomyomas from four HLRCC patients and three FH-deficient sporadic tumours and their corresponding normal myometrial tissue samples were analysed together with 58 uterine leiomyomas and corresponding normal samples from which we had both expression and whole-genome sequencing data in-house (19 *MED12* mutation-positive and 39 *MED12* and *FH* wild-type tumours) (Mehine *et al*, 2013, 2016). Gene expression data was constructed using Affymetrix GeneChip Human Exon 1.0 ST Arrays (Affymetrix) at the Biomedicum Functional Genomics Unit, Helsinki, Finland. The expression data was analysed with Partek Genomic Suite v. 6.5 (Partek Incorporated, St Louis, MO, USA) using re-mapped Brainarray Custom CDF files (HuEx10stv2_Hs_ENSG, Version 16). All samples were quantile-normalised by the Robust Multichip Average method and adjusted for probe sequence and GC-content. Unsupervised hierarchical clustering analysis (Cosine dissimilarity) was performed with 1% most variable genes ($n = 372$), defined by the coefficient of variation calculated across all tumour samples.

Statistical analyses. R software, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria, www.r-project.org), and Python, version 2.7 (Python Software Foundation, Wilmington, DE, USA, www.python.org), were utilised for statistical analyses. Differences in the frequencies of *MED12* mutations and biallelic *FH* inactivation between uterine leiomyomas from HLRCC patients and sporadic conventional uterine leiomyomas were calculated using Fisher's exact test (two-sided *P*-value). Mutual exclusiveness of *MED12* mutations and FH deficiency was evaluated utilising a permutation test. A total of $n = 1\,000\,000$ permutations were performed and mutations were randomly redistributed between samples in each permutation. Empirical *P*-value was computed as $P = (1 + k)/n$, where k is the number of permutations where at least one sample presented both *MED12* mutation and FH deficiency. The statistical significance of many *MED12* mutation-positive tumours co-occurring in HLRCC patient My31 was evaluated similarly as above by randomly redistributing tumours to patients ($n = 10\,000\,000$ permutations). Empirical *P*-value was then calculated as $P = (1 + k)/n$, where k is the number of permutations where at least one patient with six or more *MED12* mutation-positive tumours and no FH-deficient tumours was observed.

RESULTS

Mutation screening. One hundred and twenty-two uterine leiomyomas from 27 HLRCC patients were screened for somatic mutations in exons 1 and 2 of *MED12*. Of these, 34 tumours had been previously screened for exon 2 mutations (Mäkinen *et al*, 2013). Both exons were successfully sequenced in 83 FFPE samples and in all 33 fresh frozen samples (116/122; 95%). The quality of DNA obtained from six FFPE blocks of patient D6 (dating back to year 1979) was not sufficient for sequencing, and these samples were excluded from mutation screening.

Altogether nine tumours from four different HLRCC patients had *MED12* mutations (9/116; 7.8%) (Supplementary Table 2). Patients B3, B7, and E1 had one *MED12* mutation-positive tumour each, while the majority of their tumours were *MED12* wild type (1/3, 1/6, and 1/6, respectively) (Table 1). Surprisingly, all six uterine leiomyomas from patient My31 harboured different *MED12* mutation. Coding exons of *FH* were sequenced from all these six tumours, but no second hit was observed (Table 1 and Figure 1). Occurrence of this many sporadic *MED12* mutation-positive lesions in HLRCC patient is highly unlikely ($P = 2 \times 10^{-7}$, permutation test with 10 000 000 permutations).

Sixty-four sporadic conventional uterine leiomyomas (64/66; 97%) were successfully sequenced for *MED12* exons 1 and 2 (45 reported previously in (Mäkinen *et al*, 2013). Of these, 35/64 (55%) harboured a somatic *MED12* mutation (Supplementary Table 3).

Immunohistochemistry. All 122 HLRCC-associated uterine leiomyomas and 66 sporadic tumours were successfully analysed for FH deficiency utilising 2SC immunohistochemistry. Positive staining indicating biallelic *FH* inactivation was detected in 113 uterine leiomyomas from HLRCC patients (113/122; 92.6%, including six tumours from patient D6 excluded from *MED12* mutation screening). Nine uterine leiomyomas (one tumour from patients B3, B7, and E1 each, and all six tumours from My31) displayed negative 2SC immunostaining implicating the other *FH* allele to be intact and tumours to be proficient for FH activity (9/122; 7.4%; Figure 2 and Supplementary Table 2). These same tumours were observed to harbour somatic *MED12* mutations in the mutation screening (Table 1). All 66 sporadic tumours showed negative 2SC staining (Supplementary Table 3).

Based on mutation screening and results from 2SC immunohistochemistry, the difference in *MED12* mutation and biallelic *FH* inactivation frequencies between uterine leiomyomas from HLRCC patients and sporadic conventional uterine leiomyomas is highly significant ($P < 2.2 \times 10^{-16}$). Data also shows these events to be mutually exclusive ($P = 1 \times 10^{-6}$, permutation test with 1 000 000 permutations).

Table 1. *FH* and *MED12* mutation status of 21 uterine leiomyomas from four HLRCC patients with at least one *MED12* mutation-positive tumour

Patient	Tissue material	Germ-line *FH* mutation	*MED12* mutation status	2SC IHC
B3	FFPE	c.671_672delAG, p.E224fs	Wt	+
	FFPE	c.671_672delAG, p.E224fs	c.131G>A, p.G44D	–
	FFPE	c.671_672delAG, p.E224fs	Wt	+
B7	Fresh frozen	c.671_672delAG, p.E224fs	c.113-160del48, p.A38_G53del	–
	Fresh frozen	c.671_672delAG, p.E224fs	Wt	+
	Fresh frozen	c.671_672delAG, p.E224fs	Wt	+
	Fresh frozen	c.671_672delAG, p.E224fs	Wt	+
	Fresh frozen	c.671_672delAG, p.E224fs	Wt	+
	Fresh frozen	c.671_672delAG, p.E224fs	Wt	+
E1	FFPE	c.587A>G, p.H196R	Wt	+
	FFPE	c.587A>G, p.H196R	Wt	+
	FFPE	c.587A>G, p.H196R	Wt	+
	FFPE	c.587A>G, p.H196R	Wt	+
	FFPE	c.587A>G, p.H196R	Wt	+
	FFPE	c.587A>G, p.H196R	c.130G>A, p.G44S	–
My31	Fresh frozen	c.1439C>G, p. S480X	c.130G>A, p.G44S	–
	Fresh frozen	c.1439C>G, p. S480X	c.131G>T, p.G44V	–
	Fresh frozen	c.1439C>G, p. S480X	IVS1-1_139del41	–
	Fresh frozen	c.1439C>G, p. S480X	IVS1-8T>A, p.E33_D34insPQ	–
	Fresh frozen	c.1439C>G, p. S480X	c.131G>A, p.G44D	–
	Fresh frozen	c.1439C>G, p. S480X	c.130G>C, p.G44R	–

Abbreviations: *FH* = fumarate hydratase; FFPE = formalin-fixed paraffin-embedded; HLRCC = hereditary leiomyomatosis and renal cell cancer; IHC = immunohistochemistry; *MED12* = mediator complex subunit 12; wt = wild type.

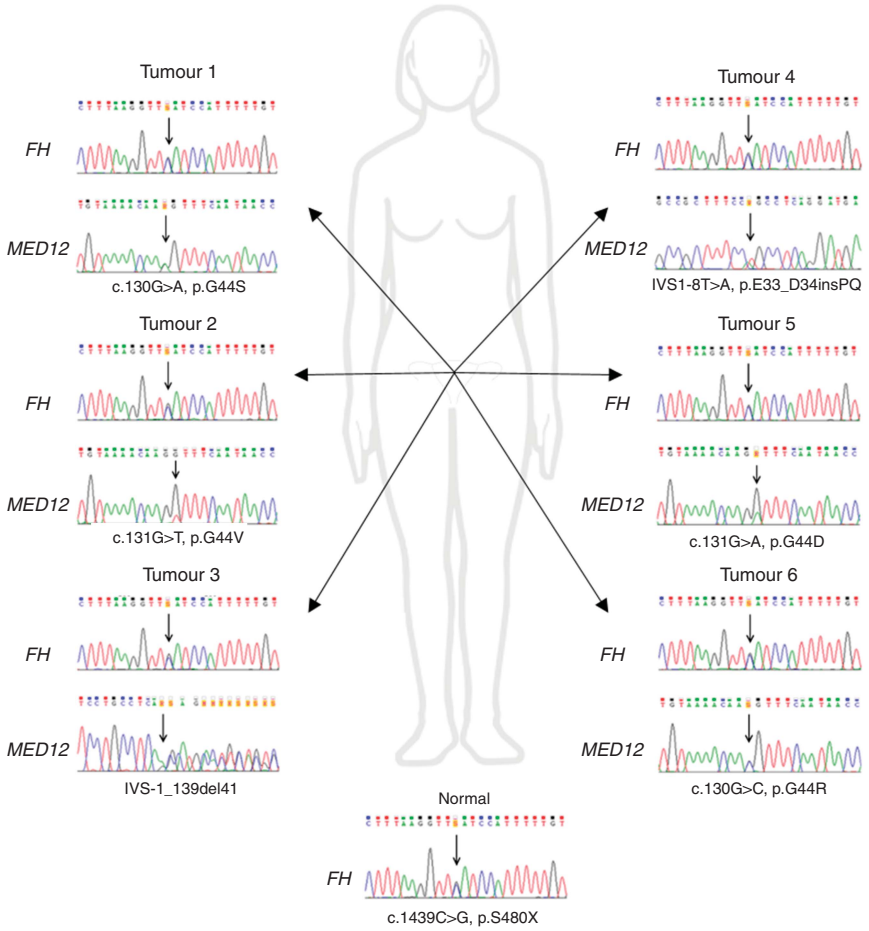

Figure 1. *FH* and *MED12* mutation status of six uterine leiomyomas form HLRCC patient My31. All six fresh frozen tumour samples retain heterozygosity at the *FH* locus and display different somatic *MED12* mutation.

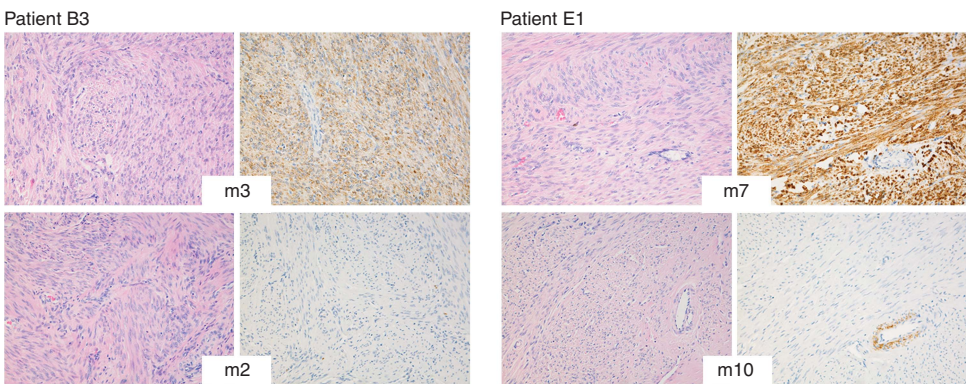

Figure 2. Haematoxylin-eosin staining and 2SC immunohistochemical staining of four uterine leiomyomas from two HLRCC patients (original magnification, × 200). Tumours deficient for FH and without a *MED12* mutation (top panel; patient B3 tumour m3 and patient E1 tumour m7) display clear positive 2SC staining, whereas tumours harbouring a *MED12* mutation and not displaying biallelic *FH* inactivation (bottom panel; patient B3 tumour m2 and patient E1 tumour m10) are negative.

Gene expression profiling. Gene expression profiling of 26 *MED12* mutation-positive, 10 FH-deficient, and 39 *MED12* and *FH* wild-type tumours and their corresponding normal myometrium tissue samples revealed that the majority of leiomyomas clustered according to the mutation status of *FH* and *MED12* (Figure 3). Leiomyomas harbouring both a germ-line *FH* mutation and a somatic *MED12* mutation displayed expression signatures similar to those with only a *MED12* mutation, instead of clustering with the FH-deficient leiomyomas. All myometrium tissue samples clustered together regardless of their germ-line mutation status.

DISCUSSION

Most uterine leiomyomas develop sporadically and in the majority (~70%) tumourigenesis is driven by very specific mutations in exons 1 or 2 of *MED12*. Simple chromosomal changes and chromothripsis-like, more complex chromosomal rearrangements affecting specific loci such as *HMGA2* (chromosome 12) or *COL4A5* and *COL4A6* (chromosome X), have also been suggested as driver aberrations in these tumours (Van de Ven, 1998;

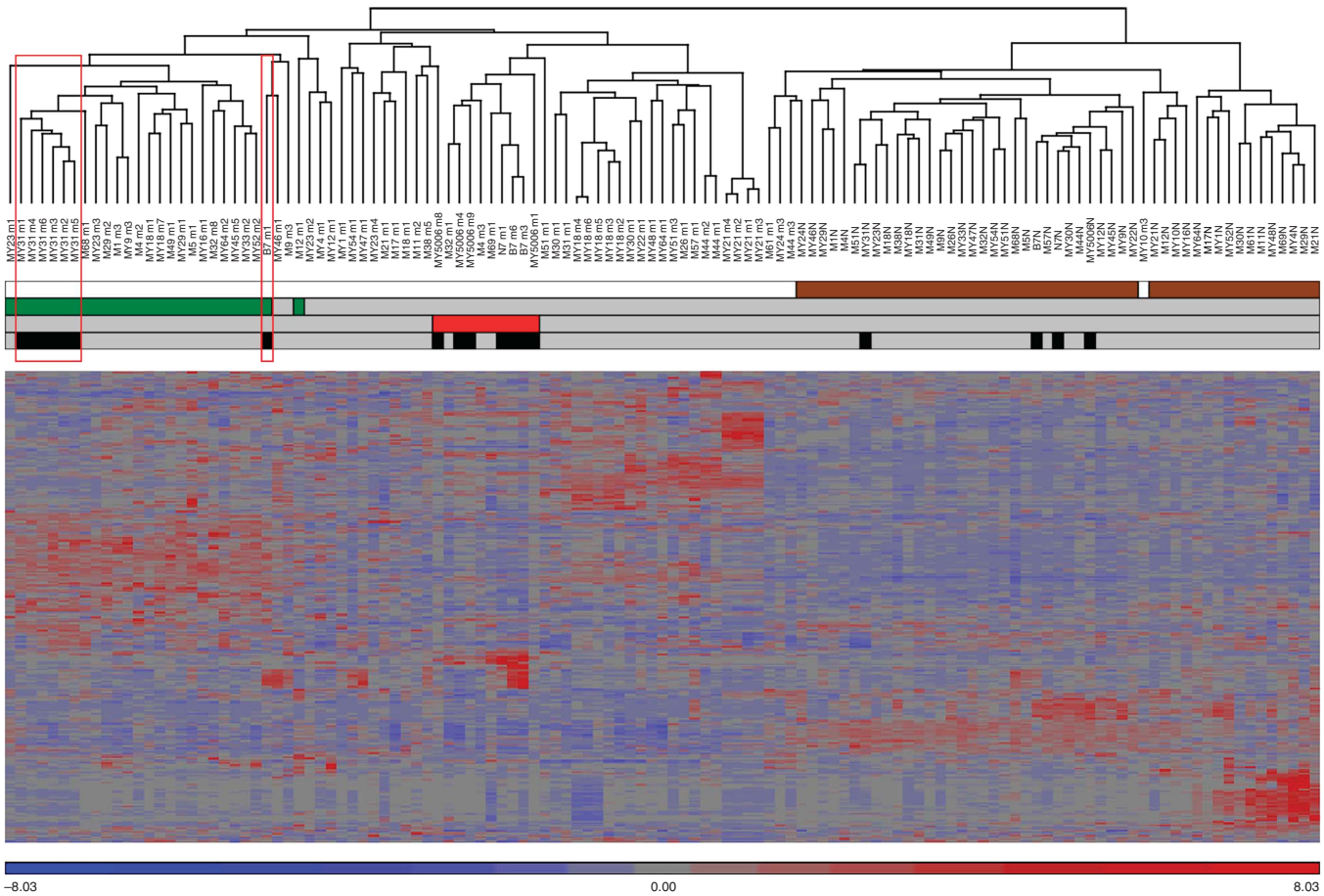

Figure 3. Unsupervised hierarchical clustering of 75 uterine leiomyomas and their corresponding normal myometrium tissue samples. Analysis included 26 *MED12* mutation-positive (green), 10 FH-deficient (red), 39 *MED12* and *FH* wild-type tumours, (grey), and 48 corresponding normal myometrium tissue samples (brown). Samples from HLRCC patients, harbouring a germ-line *FH* mutation, are marked with black. *MED12* mutation-positive and FH-deficient tumours clustered according to their mutation status. Leiomyomas with both a germ-line *FH* mutation and a somatic *MED12* mutation (My31 m1-m6 and B7 m1; red boxes) clustered with sporadic *MED12* mutation-positive leiomyomas. Germ-line *FH* mutation did not affect the clustering of normal myometrium samples, all of which clustered together.

Sandberg, 2005; Mehine *et al*, 2013). Thus far, none of these alterations have been reported to occur simultaneously in the same lesion (Markowski *et al*, 2012; Mehine *et al*, 2013; Bertsch *et al*, 2014; Mehine *et al*, 2016).

HLRCC syndrome, caused by heterozygous germ-line mutations in *FH*, is one of the few known hereditary conditions that predispose women to uterine leiomyomas. Loss of the wild-type allele leads to *FH* inactivation and subsequent tumourigenesis. To determine the frequency of *MED12* mutation-positive tumours among HLRCC patients, and to analyse whether somatic *MED12* mutations and biallelic *FH* inactivation are mutually exclusive, we performed *MED12* exons 1 and 2 mutation screening and 2SC immunohistochemical staining indicative for FH deficiency, in both HLRCC-associated and sporadic uterine leiomyomas.

In a comprehensive series of 122 HLRCC-associated uterine leiomyomas, 116 tumours were successfully analysed with both methods. The great majority of tumours (107/116; 92%) were wild type for *MED12* and showed 2SC positivity indicating them as syndrome-associated FH-deficient lesions. Twenty-three out of 27 HLRCC patients (23/27; 85%) had only FH-deficient uterine leiomyomas, with number of tumours ranging from 1 to 15 per patient. Nine uterine leiomyomas (9/116; 7.8%) from four different HLRCC patients had somatic *MED12* mutations. All these tumours displayed negative 2SC immunohistochemical staining indicating FH proficiency; these tumours are most likely sporadic. Three patients had only one *MED12* mutation-positive uterine leiomyoma in addition to several syndrome-associated tumours, while all six tumours of patient My31 were *MED12* mutation positive. Overall, these results confirm previous observations that most uterine leiomyomas in HLRCC patients are syndrome-associated and result from biallelic *FH* inactivation. Incidental sporadic tumours do, however, occur in HLRCC patients, yet these lesions account for only a small proportion of tumours. HLRCC patients tend to develop uterine leiomyomas at younger age than women with sporadic tumours (Lehtonen, 2011). Syndrome-associated tumours are also suggested to be more numerous and symptomatic, leading to hysterectomies at an earlier age, among women with HLRCC (Lehtonen, 2011). This might, to some extent, explain the rarity of sporadic lesions, which are known to reach the incidence peak towards the end of women's reproductive age. Another possible explanation is purely stochastic: as the remaining *FH* allele can be inactivated through several distinct mechanisms (missense or nonsense mutation, small insertion, or deletion leading to a shift in the reading frame, larger chromosomal aberration), it may be that one of these events happens more likely than a single, highly specific *MED12* mutation.

Altogether 66 sporadic conventional uterine leiomyomas were included in the study. Sixty-four were successfully analysed for *MED12* mutations and the majority (35/64; 55%) were mutation positive. This is in agreement with previous studies, where mutation frequencies have varied between 48 and 92% (Mehine

et al, 2014). Also the mutation spectrum with 26 highly specific missense mutations and 9 small in-frame deletions resembles previous observations. All sporadic tumours showed negative 2SC immunostaining and were thus FH proficient. This does not exclude the possibility of an underlying heterozygous germ-line *FH* mutation or the presence of one somatic alteration in some of the samples, but it shows that biallelic *FH* inactivation is a rare molecular driver event in sporadic uterine leiomyomas. This is in line with previous analyses, which have shown that the contribution of *FH* alterations on sporadic uterine leiomyomas and sporadic counterparts of other HLRCC-associated tumours is in a minor role (Kiuru *et al*, 2002; Lehtonen *et al*, 2004; Mehine *et al*, 2013; Harrison *et al*, 2015). Taken together, our results on an extensive HLRCC patients' sample series and sporadic tumours confirm the previous observations that biallelic *FH* inactivation and *MED12* mutations are mutually exclusive.

When sporadic *MED12* mutation-positive tumours were observed in HLRCC patients, these were typically individual lesions among several FH-deficient tumours. Interestingly, all six tumours from one HLRCC patient, My31, showed FH proficiency and instead of losing the wild-type *FH* allele, each tumour harboured a *MED12* mutation. All tumours displayed different *MED12* mutations thus excluding the possibility of tumour dissemination. These six tumours had been obtained at hysterectomy, which was performed when the patient was 49. She had been under surveillance due to uterine leiomyomas since the age of 28, and several lesions had been removed in two myomectomies at ages 36 and 40. We were able to collect 24 FFPE uterine leiomyoma specimens from these operations, and we analysed them with direct sequencing and 2SC immunohistochemistry. Twenty-three out of 24 specimens (96%) were considered to represent sporadic tumours due to both *MED12* mutation and negative 2SC staining. Only one tumour was wild type for *MED12* and showed positive 2SC staining, and was thus considered syndrome-associated. The germ-line *FH* mutation in patient My31 is a nonsense mutation affecting the last exon of the gene (c.1439C>G; Ser480X). The same mutation has been previously observed in another Finnish HLRCC patient with both uterine and cutaneous leiomyomas (unpublished data). This patient had a hysterectomy due to multiple leiomyomas in 1968 at the age of 31. Uterine leiomyoma samples were no longer available, but we were able to collect nine cutaneous leiomyoma samples from the pathology department's archives. All these tumours were FH deficient and thus considered syndrome-associated. In addition, other syndrome-associated nonsense and frameshift mutations have been reported in the last exon of *FH*, including one affecting Ser480 and others affecting nearby amino acids Lys477, Gly490, and Trp500 (Bayley *et al*, 2008). Intriguing and highly unlikely ($P = 2 \times 10^{-7}$) observation of several sporadic *MED12* mutation-positive lesions in HLRCC patient My31 implicates the presence of an additional genetic factor that either protects the patient from the loss of the *FH* wild-type allele or predisposes her to somatic *MED12* mutations.

Unsupervised hierarchical clustering analysis of gene expression data revealed that FH-deficient and *MED12* mutation-positive leiomyomas display distinct expression profiles. This is not surprising considering the severe metabolic stress *FH* inactivation exposes the cells to, and similar results have been reported also previously (Vanharanta *et al*, 2006; Mehine *et al*, 2013, 2016). We included both HLRCC syndrome-associated and sporadic FH-deficient tumours in the analysis, and all of these tumours clustered together irrespective of whether the inactivation had occurred through one germ line and one somatic or through two somatic mutational events. Interestingly, all six tumours of patient My31, which harbour both one germ-line *FH* mutation and a somatic *MED12* mutation, clustered together with *MED12* mutation-positive tumours and displayed expression signatures typically seen in sporadic *MED12* mutation-positive leiomyomas. Also all normal tissue samples clustered together irrespective of germ-line *FH* mutation status. These results confirm that one active *FH* allele is sufficient to maintain the metabolic balance within the cell.

To conclude, HLRCC patients' uterine leiomyomas can be formed through at least two distinct molecular mechanisms, biallelic *FH* inactivation and somatic *MED12* mutations. The great majority of HLRCC patients' tumours use the former pathway, while *MED12* mutations are the most frequent alterations in sporadic tumours. Distinct molecular mechanisms within HLRCC patients' uterine leiomyomas and sporadic tumours may affect treatment options of these patients in the future. Our results also confirm that biallelic *FH* inactivation and *MED12* mutations are mutually exclusive both within HLRCC syndrome associated and sporadic uterine leiomyomas.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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