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Uterine Fibroids in Senegal: Polymorphism of MED12 Gene and Correlation With Epidemiological Factors

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ABSTRACT

Background: Mediator complex subunit 12 (MED12) is a part of the mediator complex, which is believed to regulate transcription. Our recent study showed that MED12 is mutated at high frequency and with different mutation frequencies in Senegalese women with uterine fibroids. However, the status of mutations has not been correlated to the epidemiological factors that are implicated in uterine fibroids.

Methods: This study aimed to analyze status of MED12 mutations in Senegalese population. MED12 was sequenced in tumoral tissues and blood samples of 54 Senegalese women with uterine fibroids. Clinical and pathological data were obtained from the patient's records and other parameters were recorded. Mutation Surveyor software version 5.0.1, DnaSP version 5.10, MEGA version 7.0.26 and Arlequin version 3.5.1.3 were used to determine the level of mutations and genetics parameters. To estimate the genetic variation according to the epidemiological parameters, the index of genetic differentiation (Fst) and the genetic structure like analysis of molecular variance (AMOVA) were determined with Arlequin software version 3.5.1.3. The significance level (P-value) was 0.05.

Results: Our results showed that MED12 is mutated at 88.89% (48/54) only in tumor tissues. The variants frequencies were not similar to those found in the Finnish populations. The Chi2

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test indicates a statistically significant difference for the variants c.130G>C, c.130G>A, c.131G>A and for the Intronic Variant Site (p <0.05). This variable expression of the *MED12* gene is further confirmed by the amino acid frequency between blood and tumor tissue with a statistically significant difference for alanine, glutamic acid, lysine, methionine, threonine, valine, tryptophan and tyrosine (p <0.05). In addition, the selection test indicates that codon 44 is under positive selection (p=0.0243) in cases of uterine fibroids. Genetic diversity according to risk factors such as parity and diet was observed in uterine fibroids in Senegal (p <0.05).

Conclusion: Significant genetic diversity has been noted in uterine fibroids in Senegal. The codon 44 being under positive selection could be considered as a biomarker in uterine fibroids. Depending on the epidemiological parameters studied, parity and diet seem to be the risk factors most implicated in uterine fibroids in Senegal.

KEY WORDS: UTERINE FIBROIDS, *MED12*, MUTATIONS STATUS, RISK FACTORS, SENEGAL

INTRODUCTION

Uterine fibroids, more commonly known as myomas, are the most common benign tumors of female reproductive organs. They are associated with significant morbidity. The fibroids, which are highly variable within the uterus, develop at the expense of smooth muscle and are often separated from the myometrium by a pseudocapsule associated with connective tissue condensation (**1**). The heterogeneity of the localization of myomas and their progression in the same patient illustrates the complex biological mechanism involved in their development.

In terms of prevalence, uterine fibroids affect 20 to 25% of women in reproductive activity (**2**). Myomas affect millions of women worldwide and in 60% of cases occur in women aged 45 years (**3**). This prevalence varies by ethnicity, women of African descent having a 3- to 9-fold higher risk than Caucasian women. The only risk factors that are still best known are age, ethnicity, early age of menarche, and nulliparity (**4; 5**). Several studies have also indicated the involvement of hormonal status in the development of myomas. And according to reports published in the National Institute of Environmental Health Science-Uterine Fibroid Study (NIEHS-UFS), factors influencing steroid metabolism such as obesity, diet are associated with a risk of occurrence of uterine fibroids (**6**). Although the molecular mechanisms involved in the etiology of uterine fibroids remain incompletely elucidated, several studies have focused on the *MED12* gene (**7; 8; 9; 10**). In our previous study, the mutational penetrance of the *MED12* gene was reported in Senegalese women with uterine fibroids compared to those with breast adenofibromas (**11**). In this study, we evaluate the degree of correlation between clinicopathological parameters and the genetic evolution of uterine fibroids in Senegalese women.

MATERIALS AND METHODS

SAMPLES COLLECTION

Cancerous tissues and peripheral blood samples were collected from 54 patients with uterine fibroids (from the Military Hospital of Ouakam and General Hospital of Grand Yoff). An informed consent, written according to a standardized form has been obtained for each patient. Clinical and pathological data were recorded including age, ethnicity, age at menarche, marital status, number of pregnancies, number of child birth, hormonal contraception, diet and physical activity (Table 1). None of the patients surveyed claimed to be

taking alcohol or tobacco, which is why these factors are not included in this study.

DNA EXTRACTION AND SEQUENCING OF THE *MED12* GENE

Total DNA was extracted from patient's tissues and blood using the DNase Blood and Tissue Kit (Qiagen). The exon 2 and its flanking intronic sequence of *MED12* was amplified as previously described by Mäkinen et al. (**7**) using the forward 5′-GCCCTTTCACCTTGTTCCTT-3′ and reverse 5′-TGTCCCTATAAGTCTTCCCAACC-3′ primers. An electrophoretic migration on 1.5% agarose gel was performed to confirm the amplification. Sequencing reactions were performed in a thermal cycler MJ Research PTC-225 Peltier type with ABI PRISM BigDye TM Terminator Cycle kit. Each sample was sequenced using the forward primer. Fluorescent fragments were purified with the BigDye Xterminator purification protocol. The samples were suspended in distilled water and subjected to electrophoresis in 3730xl ABI sequencer (Applied Biosystems).

Table 1. Clinical and pathological characteristics of 54 cases analyzed

Epidemiological factors	Number of patients (%)
Age (n=36)	
≤35	11 (30.55%)
]35 – 45]	18 (50%)
>45	7 (19.45%)
Ethnicity (n=39)	
Wolof	13 (33.33%)
Sérère	4 (10.26%)
Lébou	7 (17.95%)
Bambara	3 (7.69%)
Diola	5 (12.82%)
Alpulaar	7 (17.95%)
Marital status (n=31)	
Single	8 (25,80%)
Married	20 (64,52%)
Divorced	3 (9,68%)
Age at menarche (n=18)	
≤12	1 (5.56%)
]12 – 15]	13 (72.22%)
>15	4 (22.22%)
Number of pregnancies (n=31)	
0	20 (64.51%)
I	4 (12.91%)
II	4 (12.91%)
III	1 (3.22%)
>III	2 (6.45%)
Number of childbirth (n=33)	
0	23 (69.70%)
I	7 (21.21%)
II	1 (3.03%)
III	2 (6.06%)
>III	0 (0%)
Hormonal contraception (n=23)	
Yes	2 (8.69%)

No	21 (91.31%)
Diet (n=23)	
Meat preference	7 (30.43%)
Vegetarian preference	6 (26.09%)
No preference	10 (43.48%)
Physical activity (n=23)	
Yes	5 (21.74%)
No	18 (78.26%)

MOLECULAR ANALYSIS

To determine the presence of any mutation and its relative position on *MED12* gene, a Mutation Surveyor software version 5.0.1. (www.sofgenetics.com) was used to analyze the raw sequencing data. This program can directly compare chromatograms with genomic DNA of reference sequence of *MED12* (NT_011669_70337906). The mutations found in our patients were compared to those found in the Finnish population to see if there is any different expression of the *MED12* gene.

Alignment of the sequences was carried out using the BioEdit software version 8.0.5 and ClustalW algorithm (**12**). The sequences obtained were thoroughly checked, cleaned and aligned to identify homologies among sites, and also to perform other phylogenetic analysis including the determination of variability index and genetic diversity and genetic differentiation parameters. Genetic variability parameters including the number of polymorphic sites, the total number of haplotype, the average number of nucleotide difference (K), the estimated Transition/Transversion bias (R), the amino acid frequencies and the codon test of selection were obtained through DnaSP 5.10 software and MEGA 7.0.26 (**13; 14**). The estimated Transition/Transversion bias (R) was estimated under the Kimura 2-parameter model. The codon selection test, obtained with MEGA7, was determined for exon 2 of *MED12* gene. For each codon, estimates of the numbers of inferred synonymous (s) and nonsynonymous (n) substitutions are presented along with the numbers of sites that are estimated to be synonymous (S) and nonsynonymous (N). These estimates are produced using the joint Maximum Likelihood (ML) reconstructions of ancestral states under a Muse-Gaut model of codon substitution and General Time Reversible model of nucleotide substitution. For estimating ML values, a tree topology was automatically computed. The test statistic dN - dS is used for detecting codons that have undergone positive selection, where dS is the number of synonymous substitutions per site (s/S) and dN is the number of nonsynonymous substitutions per site (n/N). A positive value for the test statistic indicates an overabundance of nonsynonymous substitutions. In this case, the probability of rejecting the null hypothesis of neutral evolution (P-value) is calculated. Values of P less than 0.05 are considered significant at a 5% level and are highlighted. Normalized dN - dS for the test statistic is obtained using the total number of substitutions in the tree (measured in expected substitutions per site). It is useful for making comparisons across data sets. Maximum Likelihood computations of dN and dS were conducted using HyPhy software package.

To estimate the genetic variation according to the epidemiological parameters, the factor of genetic differentiation (Fst) and the analysis of molecular variance (AMOVA) were determined with Arlequin software version 3.5.1.3 (**15**). Values of P less than 0.05 are considered significant at a 5% level.

RESULTS

MED12 VARIATIONS AND FREQUENCIES

The analysis of the chromatograms showed the presence of variants in the *MED12* gene for tumoral tissues. For blood samples, no variants

of the *MED12* gene were observed. Then, comparison of the variants found by Makinen et al. (2011) with those found in our study shows a variable expressivity of the *MED12* gene with mutations specific to each population (Table 2). The Chi2 test indicates a statistically significant difference for the variants c.130G>C, c.130G>A, c.131G>A and for the Intronic Variant Site (IVS). For the latter, the variants found among the Senegalese population are: IVS 204 + 77T>C; IVS 204 + 76C>T and IVS 1285_1286delA.

Most variations found in exon 2 of *MED12* were heterozygous single nucleotide polymorphisms (SNPs) (Figure 1).

Table 2. *MED12* variation status in different ethnic groups

Region	Mutations	Predicted protein change	Mutations frequencies (%)		*P-value* (Chi 2 test)
			Makinen	Our study	
Exon 2	c.107T>G	p.L36R	4.8 (11/225)	1.8 (1/54)	0.0678
Exon 2	c.128A>C	p.Q43P	1.3 (3/225)	1.8 (1/54)	0.5527
Exon 2	c.130G>C	p.G44R	7.1 (16/225)	3.7 (3/54)	0.0324
Exon 2	c.130G>A	p.G44S	7.6 (17/225)	16.6 (9/54)	<0.0001
Exon 2	c.130G>T	p.G44C	3.1 (7/225)	3.7 (3/54)	0.6573
Exon 2	c.131G>C	p.G44A	4.8 (11/225)	1.8 (1/54)	0.0678
Exon 2	c.131G>A	p.G44D	20.9 (47/225)	12.9 (7/54)	<0.0001
Exon 2	c.131G>T	p.G44V	5.3 (12/225)	7.4 (4/54)	0.2751
Exon 2	c.179A>T	p.K60M	0 (0/225)	1.8 (1/54)	0.4062
Exon 2	c.181 A>T	p.N61V	0 (0/225)	1.8 (1/54)	0.4062
[1]IVS	[2]Indels	Loss of splice acceptor	6.2 (14/225)	22.2 (12/54)	<0.0001
Exon 2	c.103_138del36	p.E35_N46del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.105_119del15	p.E35_N460delinsD	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.106_108del3	p.L36del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.110_118del9	p.T37_L39del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.112_138del27	p.A38_N46del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.114_138del25	p.L39_N46del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.115_141del27	p.L39_N47del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.116_122del7insC	p.L39_V41delinsS	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.116_154del39	p.L39_V51del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.118_119ins9	p.L39_N40insTAL	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.121_138del18	p.V41_N46del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.122_157del36	p.V41_S52del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.123_134del12	p.K42_F45del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.126_140del15	p.K42_N46del	0.9 (2/225)	0 (0/54)	0.9510
Exon 2	c.128_145del18	p.Q43_Q48del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.129_131del3	p.Q43_G44delinsH	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.133_150del18	p.F45_A50del	0.9 (2/225)	0 (0/54)	0.9510
Exon 2	c.138_152del15	p.N47_V51del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.141_161del21	p.Q48_D43del	0.4 (1/225)	0 (0/54)	0.4454
Exon 2	c.101_139del39	p.D34_N46del	0 (0/225)	1.8 (1/54)	0.4062
Exon 2	c.108_148del41	p.L36_A50del	0 (0/225)	1.8 (1/54)	0.4062

[1] IVS = Intronic Variant Site

[2] Indels = Insertion-Deletions

Exon 2	c.126_134del9	p.K42_F45del	0 (0/225)	1.8 (1/54)	0.4062
Exon 2	c.126_149del24	p.K42_A50del	0 (0/225)	1.8 (1/54)	0.4062
Exon 2	c.130_132del3	p.G44del	0 (0/225)	1.8 (1/54)	0.4062
Exon 2	c.131_154del24	p.G44_V51del	0 (0/225)	1.8 (1/54)	0.4062
Exon 2	c.138_161del24	p.N46_D43del	0 (0/225)	1.8 (1/54)	0.4062
Exon 2	c.139_145del7	p.N46_Q48del	0 (0/225)	1.8 (1/54)	0.4062

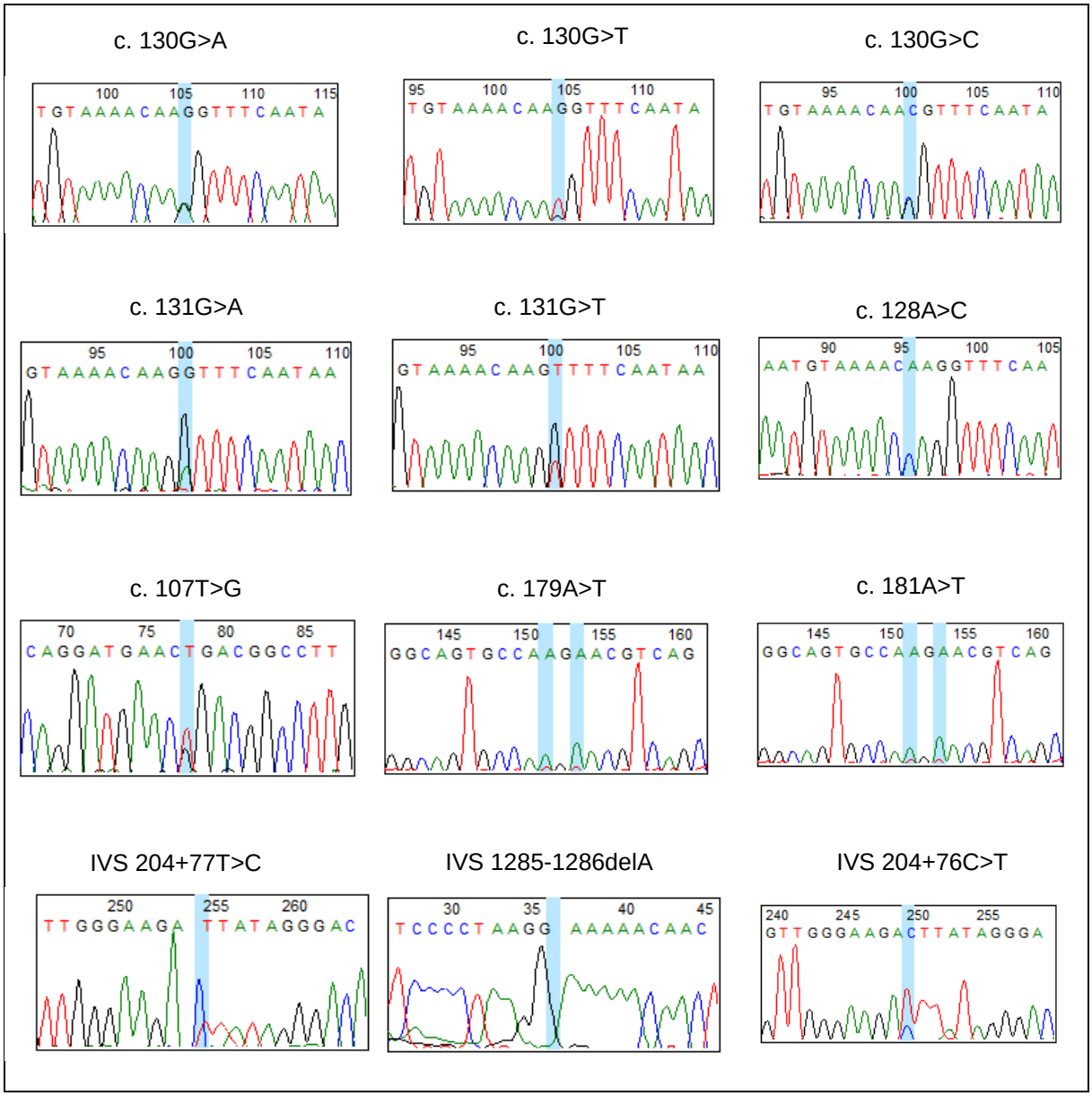

Figure 1. Heterozygous SNPs and intronic variant site of *MED12* gene

INDEX OF VARIABILITY AND GENETIC DIVERSITY

The analysis of genetic analysis in blood samples and tumor tissues indicates that only MED12 is mutated in tumor tissues (Table 3).

For the amino acid frequency (Table 4), a statistically significant difference between blood and tumor tissue was noted for alanine, glutamic acid, lysine, methionine, threonine, valine, tryptophan and tyrosine ($p < 0.05$).

The codon selection test indicates a positive selection of codon 44 *MED12* exon 2 gene ($p = 0.024319168$) characteristic of superiority of

nonsynonymous mutations compared to synonymous mutations in uterine fibroids (Table 5). In other words, all the mutations affecting the codon 44 which corresponds to glycine induce a change of the latter by another amino acid.

Depending on the epidemiological parameters studied, a variable expressivity of *MED12* gene is noted (Table 6). Although the probability values are not statistically significant, we observe a greater genetic differentiation in patients, whose age is between 35 and 45 years; Sérère, Bambara and Diola; divorced; those with early menarche; patients with multi pregnancies and those taking a contraceptive method. According to parity and diet, we note a strong genetic differentiation in each subgroup. According to inter-subgroup comparisons for each epidemiological parameter studied, genetic differentiation is greater between patients aged 35 and over; between Sérère and the Diola; between singles and divorced; those more than 3 deliveries and those with preferential meat and vegetarian diet (Table 6). With regard to menarche age, hormonal contraception and physical activity, no genetic differentiation was noted between the subgroups. This attests to the strong heterogeneity of the expression of the *MED12* gene in the case of uterine fibroids.

Table 3. Index of variability and genetic diversity

Variability index		
	Blood	Tumour tissue
Number o sites	226	226
Monomorphic sites	226	148
Polymorphic sites	0	78
Total number of mutations	0	79
Average number of nucleotide difference (K)	0	8.16401
Transition/Transversion bias (R)	0.548	1.679
Genetic diversity index		
Pi (variance)	0 (0)	0.00892 ± 0.00370
Hd (variance)	0 (0)	0.97820 ± 0.00337

Table 4. Amino acid frequencies of *MED12* exon 2 between blood ant tumor tissues samples

Amino acid	Blood	Tumour tissues	Chi2 test (P-value)
Alanine (Ala)	7.936	7.921	1.125^{E}-4 (p=0.0169)
Cysteine (Cys)	1.587	1.675	0.004 (p=0.0992)
Aspartic acid (Asn)	3.172	3.412	0.028 (p=0.2673)
Glutamic acid (Glu)	4.761	4.783	2.420^{E}-4 (p=0.0248)
Phenylalanine (Phe)	3.174	3.382	0.022 (p=0.2339)
Glycine (Gly)	14.285	13.619	0.222 (p=0.7246)
Histidine (His)	1.587	1.645	0.002 (p=0.0654)
Isoleucine (Ile)	1.587	1.645	0.002 (p=0.0654)
Lysine (Lys)	9.523	9.536	8.450^{E}-5 (p=0.0147)
Leucine (Leu)	9.523	8.927	0.178 (p=0.6531)
Methionine (Met)	0	0.030	4.500^{E}-4 (p=0.038)
Asparagine (Asn)	9.523	9.323	0.020 (p=0.2249)
Proline (Pro)	4.761	5.027	0.035 (p=0.2984)
Glutamine (Gln)	6.349	6.276	0.003 (p=0.0823)
Arginine (Arg)	6.349	6.520	0.015 (p=0.1925)

Serine (Ser)	6.349	6.703	0.063 (p=0.3953)
Threonine (Thr)	1.587	1.553	0.001 (p=0.0384)
Valine (Val)	6.349	6.368	1.805^{E}-4 (p=0.0214)
Tryptophan (Trp)	1.587	1.614	3.645^{E}-4 (p=0.0305)
Tyrosine (Tyr)	0	0.030	4.500^{E}-4 (p=0.038)

Table 5. Maximum likelihood analysis of the codon selection test of exon 2 *MED12* gene

N° Codon	Triplet	dS	dN	dN-dS	P-value
1	GAT	0	0	0	N/A
2	GAA	0.009563704	0.003429914	-0.00613379	0.998807314
3	CTG	0	0.617779466	0.617779466	0.539566891
4	ACG	1.000000039	0	-1.00000003	1
5	GCC	0	0	0	N/A
6	TTG	0	0	0	N/A
7	AAT	0	0	0	N/A
8	GTA	0	0	0	N/A
9	AAA	0	0	0	N/A
10	CAA	0	0	0	N/A
11	**GGT (p. 44Gly)**	**0.013053924**	**6.109314898**	**6.096260974**	**0.024319168**
12	TTC	0	0	0	N/A
13	AAT	0.009537585	0.001405573	-0.00813201	0.999720841
14	AAC	0.011494031	0.008011358	-0.00348267	0.997409506
15	CAG	0	0	0	N/A
16	CCT	0	0	0	N/A
17	GCT	0	0.010800866	0.010800866	0.991976761
18	GTC	0	0.003015279	0.003015279	0.997753645
19	TCT	0	0.002830321	0.002830321	0.997891291
20	GGG	0	0	0	N/A
21	GAT	0	0.003486089	0.003486089	0.999307574
22	GAG	0	0	0	N/A
23	CAT	0	0	0	N/A
24	GGC	0	0	0	N/A
25	AGT	0	0	0	N/A
26	GCC	0	0	0	N/A
27	AAG	0	0.57865028	0.57865028	0.665663532
28	AAC	0	0.384454984	0.384454984	0.873450741
29	GTC	0	0.5	0.5	0.666666667

30	AGC	0	0	0	N/A
31	TTC	0	0	0	N/A
32	AAT	0	0	0	N/A
33	CCT	0	0	0	N/A
34	GCC	0	0	0	N/A
35	AAG	0	0	0	N/A
36	GTG	0	0	0	N/A

These Fst values are further explained by molecular variance analysis (Table 7). The analyzes show that the genetic structuring of uterine fibroids depends only on parity and diet ($p < 0.05$). In other words, the molecular mechanisms involved in the occurrence of uterine fibroids in Senegalese women seem to be strongly correlated only with factors of predisposition such as parity and diet. Other risk factors such as age, ethnicity, marital status, age at menarche, pregnancy, contraception and physical activity are not associated with the molecular mechanisms involved in the etiology of fibroids uterine in Senegalese women.

The genetic evolution explained by the index of haplotypic (Hd) and nucleotide (Pi) diversity also shows a different evolution depending on the epidemiological parameters studied (Table 8). Rapid growth of tumor cells (high Hd and low Pi) is noted only in patients aged between 35 and 45 years; in all ethnic groups except Lebou; singles and divorced; in multiparous women and those with a meat diet. According to the menarche, the number of pregnancies, the contraception and the physical activity, the tumoral progression is strong whatever the subgroup.

Table 6. Degree of genetic differentiation of *MED12* gene in relation to the epidemiological parameters studied

Epidemiological parameters	**Genetic differntiation (Fst)**		
Groups Sub-groups	**Intra sub-group**	**Inter sub-groups**	
Age	**Fst**	**Between sub-groups**	**Fst (P-value)**
≤35	-0.01070	≤35 &]35 – 45]	-0.05746 (0.96396)
]35 – 45]	0.00129	≤35 & >45	-0.00446 (0.54955)
>45 ans	-0.03905	]35– 45] & >45	0.04612 (0.12613)
Ethnicity	**Fst**	**Between sub-groups**	**Fst (P-value)**
Wolof	-0.15637	Wolof & Sérère	-0.06858 (0.57658)
Sérère	0.05826	Wolof & Lébou	-0.05790 (0.87387)
Lébou	-0.06566	Wolof & Bambara	-0.13953 (0.84685)
Bambara	0.06273	Wolof & Diola	-0.05098 (0.84685)
Diola	0.04057	Wolof & Alpulaar	-0.07670 (0.86486)
Alpulaar	-0.15512	Sérère & Lébou	-0.06347 (0.63964)
		Sérère et Bambara	-0.23077 (0.97297)
		Sérère & Diola	0.01235 (0.36937)

		Sérère & Alpulaar	-0.02188 (0.37838)
		Lébou & Bambara	-0.15030 (0.90991)
		Lébou & Diola	-0.04239 (0.70270)
		Lébou & Alpulaar	-0.05290 (0.89189)
		Bambara & Diola	-0.06679 (0.74775)
		Bambara & Alpulaar	-0.11632 (0.35135)
		Diola & Alpulaar	-0.05807 (0.81982)
Marital status	**Fst**	**Between sub-groups**	**Fst (P-value)**
Single	-0.03845	Single & Married	0.00111 (0.35135)
Married	0.00299	Single & Divorced	0.03751 (0.20721)
Divorced	0.01886	Married & Divorced	-0.14797 (0.99099)
Age at menarche	**Fst**	**Between sub-groups**	**Fst (P-value)**
≤12	0.17497	≤12 &]12 – 15]	-0.46988 (0.50450)
]12 – 15]	-0.12550	≤12 & >15	-0.50980 (0.48649)
>15	-0.15887	] 12 – 15] & >15	-0.03581 (0.34234)
Number of pregnancy	**Fst**	**Between sub-groups**	**Fst (P-value)**
0	-0.02989	0 & I	-0.01314 (0,25225)
I	-0.01230	0 & II	0.08175 (0,18018)
II	-0.20002	0 & III	-0.65090 (0,99099)
III	0.21494	0 & >III	-0.09167 (0,13514)
>III	0.17542	I & II	0.00383 (0,65766)
		I & III	-0.61404 (0,99099)
		I & >III	0.04306 (0,38739)
		II & III	-0.75000 (0,99099)
		II & >III	-0.03529 (0,24324)
		III & >III	0.42857 (0,27027)

Table 6. Degree of genetic differentiation of the *MED12* gene in relation to the epidemiological parameters studied

Epidemiological parameters	**Genetic differentiation (Fst)**		
Groups Sub-groups	**Intra sub-group**	**Inter sub-groups**	
Number of Childbirth	**Fst**	**Between sub-groups**	**Fst (*P-value*)**
0	0.14950	0 & I	-0.02878 (0.59459)
I	0.22737	0 & II	0.66210 (0.99099)
II	0.34174	0 & III	-0.17995 (0.75676)
III	0.31084	I & II	0.75092 (0.99099)
		I & III	-0.18830 (0.90090)
		II & III	0.89286 (0.33333)
Hormonal contraception	**Fst**	**Between sub-groups**	**Fst (*P-value*)**
Yes	0.17376	Yes & No	-0.05136 (0.18919)
No	-0.07280		
Diet	**Fst**	**Between sub-groups**	**Fst (*P-value*)**
1 Meat preference	0.24962	1 & 2	0.17102 (0.10811)
2 Vegetarien preference	0.03099	1 & 3	-0.04763 (0.92793)
3 No preference	0.26587	2 & 3	0.26340 (0.01802)

Physical activity	Fst	Between sub-groups	Fst (*P-value*)
Yes	-0.00058	Oui & Non	-0.04971 (0.63063)
No	-0.06336		

Table 7. Genetic structuring of *MED12* according to epidemiological parameters

Epidemiological parameters	Source of variation	Percentage of variation (%)	Fst (*P-value*)
Age	Intra sub-group	101.02	-0.01022 (0.54448)
	Inter sub-groups	-1.02	
Ethnicity	Intra sub-group	107.57	-0.07575 (0.98925)
	Inter sub-groups	-7.7	
Marital status	Intra sub-group	100.72	-0.00718 (0.37928)
	Inter sub-groups	-0.72	
Age at menarche	Intra sub-group	111.62	-0.11622 (0.42424)
	Inter sub-groups	-11.62	
Number of pregnancies	Intra sub-group	102.84	-0.02843 (0.41642)
	Inter sub-groups	-2.84	
Number of childbirth	Intra sub-group	**81.84**	0.18162 (0.04700)
	Inter sub-groups	**18.16**	
Hormonal contraception	Intra sub-group	105.14	-0.05136 (0.24927)
	Inter sub-groups	-5.14	
Diet	Intra sub-group	**80.74**	0.19262 (0.02737)
	Inter sub-groups	**19.26**	
Physical activity	Intra sub-group	104.97	-0.04971 (0.63441)
	Inter sub-groups	-4.97	

Table 8. Heterotypic and nucleotide diversity according to epidemiological parameters

Epidemiological parameters	Index of genetic diversity	
	Hd	Pi
Age		
≤35	0.9818 ± 0.01295	0.01123 ± 0.00922
]35 - 45]	0.9739 ± 0.00857	0.00684 ± 0.00078
>45	1 ± 0.00912	0.01180 ± 0.00127
Ethnicity		
Wolof	1 ± 0.01246	0.00665 ± 0.00058
Sérère	1 ± 0.03125	0.00885 ± 0.00072
Lébou	1 ± 0.00912	0.01054 ± 0.00126
Bambara	1 ± 0.07407	0.00887 ± 0.00088
Diola	1 ± 0.02592	0.00889 ± 0.00090
Alpulaar	0.9524 ± 0.04351	0.00717 ± 0.00073
Marital status		
Single	0.9643 ± 0.00893	0.00674 ± 0.00053
Married	0.9947 ± 0.00298	0.01058 ± 0.00124
Divorced	1 ± 0.09817	0.00737 ± 0.00073
Age at menarche		
≤12	N/A	N/A
] 12 - 15]	0.9872 ± 0.00788	0.00994 ± 0.00086

>15	1 ± 0.07031	0.00516 ± 0.00053
Number of pregnancies		
0	0.9684 ± 0,00817	0.00867 ± 0.00088
I	1 ± 0.04948	0.00737 ± 0.00072
II	1 ± 0.03125	0.00885 ± 0.00086
III	N/A	N/A
>III	1 ± 0.2500	0.00440 ± 0.00040
Number of childbirth		
0	0.9723 ± 0.00801	0.00865 ± 0.00084
I	1 ± 0.01067	0.00925 ± 0.00110
II	N/A	N/A
III	1 ± 0.2500	0.01327 ± 0.00121
Hormonal contraception		
Yes	1 ± 0.2500	0.00440 ± 0.00040
No	0.9762 ± 0.00681	0.00905 ± 0.00087
Diet		
Meat preference	0.9048 ± 0.01686	0.00759 ± 0.00090
Vegetarian preference	1 ± 0.00455	0.01018 ± 0.00841
No preference	1 ± 0.00200	0.01082 ± 0.00938
Physical activity		
Yes	1 ± 0.02594	0.00946 ± 0.00091
No	0.9673 ± 0.00683	0.00973 ± 0.00062

DISCUSSION

In the present study, *MED12* gene, which is a nuclear gene involved in transcriptional regulation, has been investigated in Senegalese women with uterine fibroids. In each patient, a blood sample (to serve as a control) and a tumor sample were taken. Analysis of the chromatograms revealed a presence of mutations only at tumor tissue with a frequency of 88.89%. This confirms the hypothesis that *MED12* is involved in the occurrence of uterine fibroids. The link between *MED12* mutations and fibroids was first described by Mäkinen et al. (2011) and according to him, *MED12* mutations represent the largest genetic defect of uterine fibroids. In addition, *MED12* mutations in tumors other than uterine fibroids are rare. Only 0.3 to 0.5% of colorectal cancers have *MED12* mutations, stating that they are only transient mutations (**16; 17**). 5% of prostate cancers have different *MED12* mutations (**18; 19**). No *MED12* mutation has been detected in malignant breast and ovarian tumors or any other carcinoma. The involvement of *MED12* in uterine fibroids could be explained by the fact that it is a gene that plays a significant role in various cell signaling mechanisms by interacting with multiple receptors, particularly estrogen receptors (**20**). And it is now accepted that uterine fibroids are dependent on steroid hormones including estrogen. Indeed, estrogens are considered the main agent inducing the growth of uterine fibroids. These findings are that uterine fibroids are rare during pubertal age, they increase or show minimal changes during reproductive age, and generally decline during postmenopausal period. In addition, studies have shown an accumulation of estrogen receptors in fibrotic tissues such as uterine fibroids (**21; 22; 23**).

Although the involvement of *MED12* gene in uterine fibroids is well established, the heterogeneity of the frequency of mutations found in comparison with those found by Mäkinen (2011) raises questions. A statistically

significant difference was noted for variants c.130G> C, c.130G> A, c.131G> A and for Intronic Variant Site ($p < 0.05$). This difference could explain the disparity in the occurrence of fibroids between Caucasian and Black women. The effect of race on the incidence and severity of uterine fibroids is particularly significant. Marsh et al. (2013) conducting a prospective pilot study in young black and Caucasian women (18-30 years old) with uterine fibroids demonstrated a prevalence 3 times higher in black women compared to Caucasian women (26% vs. 7%) (**24**). Nevertheless, the causes of racial disparity are still unknown because they have long been attributed to a difference in socio-economic status, access to care and environmental mechanisms. It would be interesting to focus on these variants of *MED12*.

The variable expressivity of *MED12* gene is further confirmed by the distribution of the amino acid frequency between blood and tumor tissue with a statistically significant difference for alanine, glutamic acid, lysine, methionine, threonine, valine , tryptophan and tyrosine ($p < 0.05$). Particular emphasis is placed on the methionine which is considered as the initiator codon of the protein translation. This observation is the fact that methionine is absent for blood samples (0) and present in tumor tissues (0.030). This difference could have a crucial role on the functional mechanism of the Med12 protein by causing its activation in tumor cells. Indeed, with Med13, cdk8 and cyclin c, Med12 forms a sub-complex known as the kinase or CDK8 module. This complex is suggested to have a role in transcriptional repression but also seems to act as a co-regulator. By mass spectrometry and western blot, Turunen et al. (2014) demonstrated that the mutant *MED12* showed affinity for CDK8 and cyclin C compared to the wild-type allele (**25**). The consequence is the absence of the inhibition of transcription. This lack of transcription inhibition could be explained by the presence of methionine in tumor tissues.

The role of *MED12* in the occurrence of uterine fibroids is also to be investigated with codon 44 of exon 2 because it is the only codon under positive selection in tumor tissues. In other words, all the mutations affecting codon 44 cause an amino acid change and therefore an aberrant function of the med12 protein. A study by Bourbon et al., Involving 39 different species, showed that codon 44 is the most conserved codon of *MED12* gene, which states that this codon plays an important role in the normal function of the protein. Moreover, according to the work of Turunen et al. (2014), the binding domain of cyclin C resides at the N-terminal region encoded by exons 1 and 2 of *MED12* gene and codon 44 would play a role in this membership (**25**). This further confirms the transcriptional activation of the med12 aberrant function in uterine fibroids and that codon 44 is essential for this process. It would be interesting to investigate this codon for therapeutic perspectives.

Despite the progress of research on the etiology of myomas, the factors that initiate their pathogenicity remain incompletely elucidated both in Africa and in the rest of the world. Among all the epidemiological parameters taken into account in this study, only parity and diet seem to be genetically correlated with the occurrence of uterine fibroids in Senegalese women. No genetic structuring was observed as a function of age, ethnicity, marital status, age at menarche, pregnancy, contraception and physical activity. The heterogeneity of the predisposing factors in uterine fibroids illustrates the complex biological mechanism involved in their development. Indeed all epidemiological studies have revealed an inverse association between parity and uterine fibroids suggesting a protective effect of childbirth on the occurrence of myomas (**2; 26**). According to Marshall et al. (1998), there is a reduction in the prevalence of uterine fibroids in multiparous and late menarche women (**27**). The complicated interpretations of these observations are the fact that steroid hormone levels are very high during

pregnancy and paradoxically fibroids may decrease in size. These different observations on the effect of parity and the occurrence of uterine fibroids between Senegalese women and other populations could be explained by a difference in the biosynthesis or metabolism of estrogen or by environmental mechanisms such as lifestyle. Indeed a positive correlation has been noted between diet and genetic expression of *MED12* in cases of uterine fibroids. Genetic differentiation is more observed in patients with a meat preference. Recently, Wise and Laughlin-Tommaso (2016) published the results on the relationship between dietary fat intake and myoma risk in African-American women, confirming an increased risk associated with the consumption of omega-3 fatty acids (**28**). They validated the hypothesis that a diet rich in fruits and vegetables reduced the risk. Indeed according to the studies of Chiaffarino et al. (1999), women with uterine fibroids are more likely to consume beef, other red meats and ham and have less frequent consumption of green vegetables, fruits and fish (**29**). The multivariate odds ratios were 1.7 for beef and other red meats, 1.3 for ham and 0.8 for fruit consumption. The limitation of this current diet study is the absence of data on total energy intake as information was collected only on the frequency of consumption of vegetables compared to red meat and in patient interviews. Further research would be interesting to evaluate the effect of delivery and fat intake on the biology of uterine fibroids.

According to the tumor progression, the genetic evolution explained by the haplotype and nucleotide diversity index also shows a different evolution depending on the epidemiological parameters studied. Rapid growth of tumor cells, characterized by high haplotypic diversity (Hd) and low nucleotide diversity (Pi) is noted only in patients aged]35 - 45]; in all ethnic groups except Lebou; singles and divorced; in women with 3 childbirth and in those with a meat diet. According to the menarche, the number of childbirth, the contraception and the physical activity, the tumoral progression is strong whatever the subgroup. These observations confirm the heterogeneity of uterine fibroids. Histologically, there are mitotically active fibroids, hemorrhagic cell fibroids, atypical or bizarre fibroids and epitheloid fibroids, and the genetic mechanism involved in each of these subtypes differs. It would be interesting to include subtypes in the clinical record to better understand the parameters involved in the molecular mechanism of myomas.

CONCLUSION

At the end of our work, the results obtained show for the first time a genetic structuring of uterine fibroids as a function of parity and diet; beyond confirming the involvement of *MED12* particular gene codon 44 of exon 2 in the occurrence of uterine fibroids among Senegalese women. In addition to this, the results open avenues for understanding the mechanisms involved in racial variation in the prevalence of uterine fibroids as well as predisposing factors. View the accepted results, it is clear that more research is needed to determine the risk factors associated with the development and growth of the myoma, because they cause significant morbidity and affect the quality of life. A clear overview of the epidemiology of myoma has not been achieved and future research on modifiable risk factors such as vegetarian diet, taking contraception among others could inform prevention myoma and provide new approaches not surgical treatment of myoma.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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