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Original Article

Mediator Complex Subunit 12 Gene Polymorphisms in Uterine Fibroids and Breast Fibroadenomas in Senegalese Women

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Mediator complex subunit 12 (*MED12*) is a part of the mediator complex, which is believed to regulate transcription. *MED12* is mutated at high frequency and with different mutation frequencies in uterine fibroids and breast fibroadenomas of different populations. This study aimed to analyze *MED12* mutations in Senegalese population. *MED12* was sequenced in the tumoral tissues and blood samples of Senegalese women with uterine fibroids or breast fibroadenomas. Surveyor software version 5.0.1, DnaSP version 5.10, MEGA version 6.06 and Arlequin version 3.5.1.3 were used to determine the level of mutations and genetics parameters. Our results showed the presence of variants in the tumoral tissues only, with most of them being heterozygous single nucleotide polymorphisms. Deletion in polyA tail was identified for the first time in the studied population. Data also showed that *MED12* exon 2 was under positive selection in case of uterine fibroids and breast fibroadenomas. The variants frequencies were not similar to those found in the Finnish or Southern United States populations for cases of uterine fibroids, and to Japanese population for cases of breast fibroadenomas. These results suggest that *MED12* variants could contribute to the development of uterine fibroids and breast fibroadenomas. The present study contributes to the current information on *MED12* variations in different populations and may aid in the development of personalized diagnoses for patients with uterine fibroids or breast fibroadenomas in the future.

Keywords: Uterine fibroid, breast fibroadenoma, *MED12* mutations.

Uterine fibroids and breast fibroadenomas are benign tumors with high fibrous component, and are among the major health challenges of women. Despite their benign nature, they are associated with high morbidity and are characterized by a high prevalence in African women, a dependence to steroid hormones, an accumulation of extracellular matrix as well as excessive and abnormal presence of collagen fibers (1-4). In a study by Baird et al., it was reported that uterine

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fibroids affect 20 - 25% of women of reproductive age and nearly 70% of women by age 50 (2). Breast fibroadenomas are the most common benign pathology of the mammary gland. They represent 50% of all biopsies of benign breast tumors with 75% increased rate of biopsies in women under 20 years (5). Recently, several independent genetic studies have suggested the involvement of genes including *MED12* in these benign fibrotic diseases (6-10).

In spite of the efforts made to understand the molecular mechanisms that govern these tumors development, some questions remain unknown: (i) why is there racial difference in the prevalence and incidence of uterine fibroids and breast fibroadenomas? (ii) is there a genetic base which could explain the similarities noted between uterine fibroids and breast fibroadenomas? Therefore in this study, we aimed to determine the involvement of the *MED12* gene in cases of uterine fibroids and breast fibroadenomas among Senegalese women by evaluating the genetic variability of *MED12* and comparing the variants found in our patients with those in other populations as well as determining the genetic evolution of both pathologies.

Materials and methods

Samples collection

Cancerous tissues and peripheral blood samples were collected from 25 patients with uterine fibroids (from the Military Hospital of Ouakam) and 25 women with breast fibroadenomas (from Aristide le Dantec Hospital). An informed consent, written according to a standardized form has been obtained for each patient. This study was approved by the Ethics switched Cheikh Anta Diop University of Dakar (UCAD).

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. (6) 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).

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).

Alignment of the sequences was carried out using the BioEdit software version 8.0.5 and ClustalW algorithm (11). 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 indices and genetic diversity, genetic differentiation parameters, mutation rate and demo-genetic evolution.

Genetic variability parameters including the number of polymorphic sites, the total number of haplotype, the average number of nucleotide difference (K), the nature of change (% transitions and transversions) and the Z test of selection for each studied subject were obtained through DnaSP 5.10 software (12) and MEGA 6.06 (13). The Z test of selection was determined for exon 2 of *MED12* gene. The hypothesis that nonsynonymous mutations are superior to synonymous mutations (dN > dS) was used to evaluate the selection of Z test for the exon 2 of the *MED12* gene under positive

selection. This test was performed using the Nei and Gojobori model and pairwise deletion method.

Genetic differentiation factor (Fst) was estimated between control individuals and patients with uterine fibroids and between control individuals and patients with breast fibroadenomas. The Fst values were obtained using the Arlequin version 3.5.1.3 program (14) and a value of $P < 0.05$ was considered as significant.

To determine the evolution of mutation rate for the uterine fibroids and breast fibroadenomas, the Network software version 4.6.1.4 was used for estimation.

The distribution analysis of disparity (Mismatch distribution) was also determined through a graphical representation of the distribution of genetic distances between individuals in a population. Analysis of mismatch was carried out and detected using the SSD (sum of squared deviations) and the raggedness (Rag irregularity index) as indices to test the quality of fit of the distribution. The graphs for uterine fibroids and breast fibroadenomas were built with the DnaSP software 5.10 (12) and SSD and Rag indices were obtained with the Arlequin 3.5.1.3 program (14).

Results

MED12 variations

The analysis of the chromatograms showed the presence of variants in the *MED12* gene for tumoral tissues (Table 1). For blood samples, no variants of the *MED12* gene were observed. Our results show for the first time the presence of a deletion at the poly-adenine (polyA) tail located within the intronic region flanking exon 2 in both uterine fibroids and breast fibroadenomas. Mutations at codon 44 of *MED12* were also frequently found (Table 1). Most variations found in exon 2 of *MED12* were heterozygous single nucleotide polymorphisms (SNPs) (Figure 1).

MED12 variations frequencies

Comparison of varitations with those found in

Table 1. Summary of somatic MED12 variations observed in uterine fibroids and breast fibroadenomas

Nucleotide change	Predicted protein change	Location
Uterine fibroids		
het1348G>T	44G>C	Exon 2
1285-1286delA		Intron1
het1349G>A	44G>D	Exon 2
het1311G>T		Intron 1
het1348G>A	44G>S	Exon 2
het1348G>C	44G>R	Exon 2
het1310T>A		Intron 1
het1351T>G	45F>V	Exon 2
Brest fibroadenomas		
het1338T>A	40N>K	Exon 2
het1335G>A	39L>L	Exon 2
1285-1286delA		Intron 1
het1332C>G	38A>A	Exon 2
het1328C>G	37T>R	Exon 2
het1349G>A	44G>D	Exon 2
het1333T>G	39L>V	Exon 2

het: heterozygous; del: deletion.

literature, revealed the presence of new variations for both uterine fibroids and breast fibroadenomas. This is the case of 1285-1286delA, 1351T>G, 1328C>G, 1331C>T, 1333T>G and 1338T>A. Moreover, variations found in other populations were not observed in the studied population (Table 2). Nevertheless, variations in positions 1348 and/or 1349 were noted to be frequently observed in all studied groups.

Indices of variability and genetic diversity

As demonstrated by the analysis of the chromatograms, the set of determined parameters indicate that there was no variability of *MED12* gene for controls as compared to tumoral tissues (Table 3). The nature of the varations indicated that transversions occurred more frequently than transitions for both uterine fibroids and breast fibroadenomas (Table 3). The Z test of selection showed a statistically significant probability (P= 0.02 and 0.01) for both uterine fibroids and breast fibroadenomas, respectively.

Estimation of genetic differentiation factor between groups showed a difference in the genetic evolution between uterine fibroids and breast fibroadenomas with a statistically significant Fst value between uterine fibroids and controls (p-value = 0.021) and no significant Fst value between breast fibroadenomas and controls (P= 0.108) (Table 4).

The estimated mutation rate for uterine fibroids and breast fibroadenomas showed a different evolution speed between the mutations, and also between the two pathologies for the same mutation (Table 5).

The graph showing mismatch indicated a difference for the two diseases with a growth signal for uterine fibroids (unimodale curve) and breast fibroadenomas that show stability (multimodale curve) (Figures 2 and 3). These graphs were supported by the SSD and Rag indices, which were not significant for uterine fibroids indicating that there was no difference between the observed and expected values.

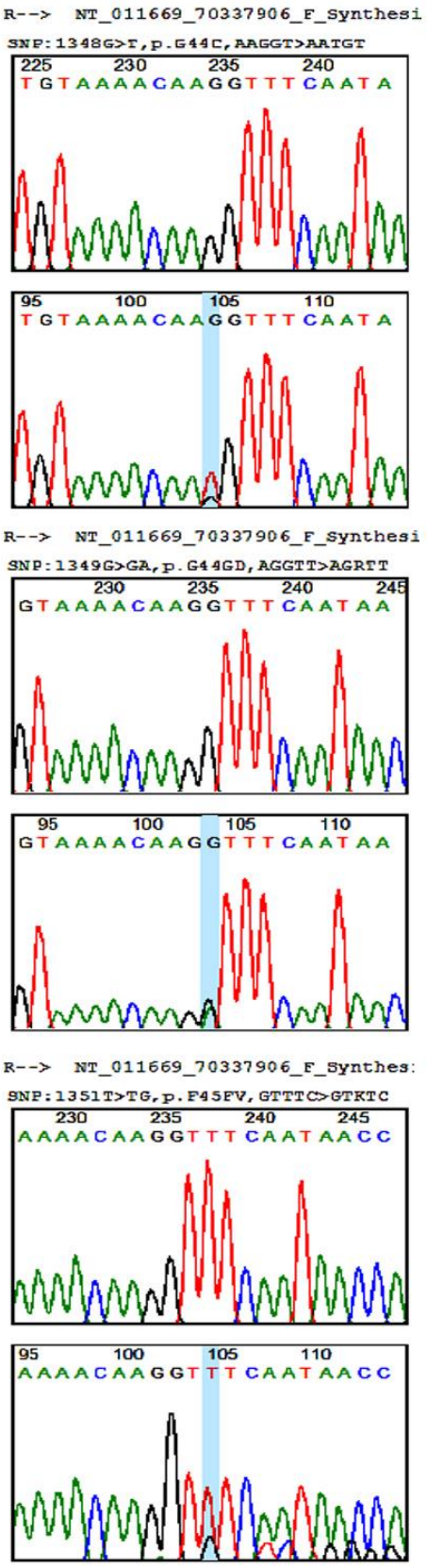

Figure 1. Heterozygous SNPs of MED12 exon 2. Top panel: 1348G>T; middle panel: 1349G>A; bottom panel: 1351T>G; R: reference sequence; F: sample sequence.

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

Uterine fibroids

Variants	Finnish population (Makinen et al., 2011)	Southern United States (Halder et al., 2015)	Present study
1285-1286 delA	**0% (0/225)**	**0% (0/143)**	**28% (7/25)**
1325T>G	4.9% (11/225)	1.4% (2/143)	0% (0/25)
1346A>C	1.3% (3/225)	1.4% (2/143)	0% (0/25)
1348G>C	7.1% (16/225)	2.8% (4/143)	4% (1/25)
1348G>A	7.5% (17/225)	6.9% (10/143)	4% (1/25)
1348G>T	3.1% (7/225)	2.8% (4/143)	4% (1/25)
1349G>C	4.9% (11/225)	1.4% (2/143)	0% (0/25)
1349G>A	20.9% (47/225)	20.2% (29/143)	16% (4/25)
1349G>T	5.3% (12/225)	1.4% (2/143)	0% (0/25)
1351T>G	**0% (0/225)**	**0% (0/143)**	**4% (1/25)**

Breast fibroadenomas

Variants	Japanese population (Nagasawa et al., 2015)	Present study
1285-1286 delA	**0%**	**32%**
1324-1347 del27	11.1%	0%
1325T>G	11.1%	0%
1328C>G	**0%**	**12%**
1331C>T	**0%**	**16%**
1333T>G	**0%**	**4%**
1338T>A	**0%**	**16%**
1340-1366 del24	11.1%	0%
1345-1350 del6	11.1%	0%
1349G>A	22.2%	12%

Del: deletion; A: adenine; G: guanine; T: thymine; C: cytosine.

Table 3. Indices of variability and genetic diversity

Parameters		NC	UF	BF
Number of sites		226	226	226
Monomorphic sites		226	214	203
Polymorphic sites		0	11	22
Number of haplotypes		1	12	16
Average number of nucleotide difference K		0	1.230	2.600
Mutations change	**Transition (%)**		35.92	29.34
	Transversion (%)		64.08	70.66
Z-test (dN > dS)			2.02 (0.02)	2.12 (0.01)

NC: negative control; UF: uterine fibroids; BF: breast fibroadenomas.

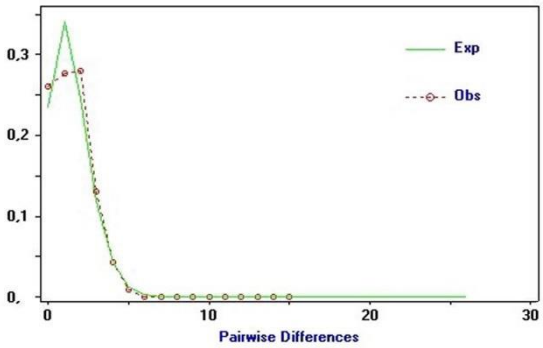

Figure 2. Mismatch distribution for uterine fibroids group. SSD= 0.0019 (P= 0.7900); Rag= 0.0210 (P= 0.5600).

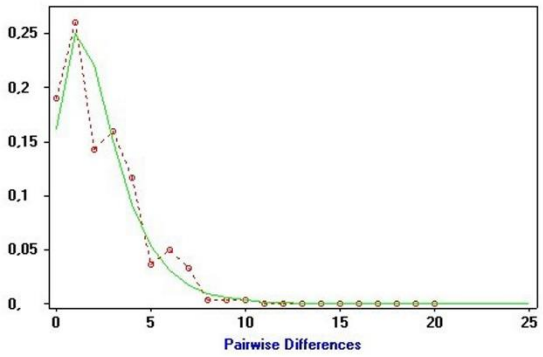

Figure 3. Mismatch distribution for breast fibroadenomas group. SSD = 0.0015 (P = 0.0200); Rag= 0.059 (P= 0.050).

Table 4. Genetic differentiation (Fst) between uterine fibroids and breast fibroadenomas for *MED12* gene

Groups	Between groups	P-value
NC	0.0425	0.021
UF		
NC	0.0329	0.108
BF		

NC: negative control; UF: uterine fibroids; BF: breast fibroadenomas.

Table 5. *MED12* mutations rate

Mutations	Uterine fibroids	Breast fibroadenomas
1285-1286 delA	0.2	0.22
1328C>G	-	0.1
1331C>T	-	0.11
1333T>G	-	0.03
1338T>A	-	0.1
1348G>T	0.033	-
1348G>A	0.033	-
1348G>C	0.033	-
1349G>A	0.121	0.027

Discussion

In the present study, the *MED12* gene, which encodes a nuclear protein involved in transcriptional regulation, was investigated in tumoral tissues of patients with uterine fibroids and breast fibroadenomas in comparison with their blood as control.

MED12 variations were observed only in tumoral tissues. Thus, these results suggest that the *MED12* gene maybe involved in the occurrence of uterine fibroids and breast fibroadenomas among Senegalese women. This observation corroborates with the study of Mäkinen et al. which showed the presence of *MED12* mutations in tumors at a prevalence of 70% in comparison with myoma tissue from normal myometrium in women in Finland (6).

Beyond this, several recent studies demonstr-

ated that mutation in exon 2 of *MED12* gene is involved in the pathogenesis of uterine fibroids (7-9, 15, 16). This implication of *MED12* in cases of breast fibroadenomas was also demonstrated in a study on Japanese women (10). The role played by this gene could be explained by the fact that it has a significant role in various cellular signaling mechanisms by interacting with multiple receptors including the estrogen receptor (17). Since uterine fibroids and breast fibroadenomas are dependent on steroid hormones including estrogen, a buildup of estrogen receptors have been demonstrated in fibrotic tissues of uterine fibroids cases (18-20).

Conspiciously, our results show for the first time the presence of a deletion located at the poly-A tail of intron 1 for both cases of uterine fibroids and breast fibroadenomas. This mutation may therefore play an important role in the severity of symptoms observed in black women compared to white women, and the deletion might influence the translation of *MED12* leading to the production of a truncated protein. Thus, further studies are necessary to determine the exact function of this region in the MED12 protein synthesis and also in the pathogenicity of uterine fibroids and breast fibroadenomas.

Most of the variations found at exon 2 affect codon 44 of *MED12* gene for cases of uterine fibroids and codons 37, 38, 39, 40 and 44 for cases of breast fibroadenomas,suggesting that codon 44 could be essential for normal functioning of the *MED12* gene. According to Turunen et al., the binding domain of cyclin C lies at the N-terminal region encoded by exons 1 and 2 of *MED12* gene and codon 44 plays a role in this binding (21).

The mutations found in these two pathologies are mostly linked to transversions (e.g. 64.08% in case of uterine fibroids and 70.66% for breast fibroadenomas). This is consistent with the general characteristics of mutations in nuclear DNA which is in a protected environment, surrounded by the nuclear envelope and subjected to a repair mechanism. The suggestion that *MED12* gene may be involved in the occurrence of uterine fibroids and breast fibroadenomas from these results is validated by the selection test. One way to test whether a gene is under positive selection is to compare the relative abundance of synonymous substitutions (mutations that do not affect the amino acid used) and non-synonymous substitutions (mutations inducing a change in amino acid) of the coding sequences of the gene. This test, indicated that exon 2 of *MED12* was under positive selection in both cases of uterine fibroids and breast fibroadenomas, with statistically significant P-values (0.02 and 0.01, respectively) confirming the greater involvement of this gene in the occurrence of these two pathologies. It would be interesting to determine the role of exon 2 of *MED12* in the synthesis of MED12 protein in patients with uterine fibroids and breast fibroadenomas.

Although *MED12* seems to be involved in these pathologies, the observed mutations appear to be heterogeneous. This could be a result of different anatomical location of the two pathologies which may lead to varying expression of *MED12* according to the histology of the tumor. This is in tandem with the study of Perot et al., who indicated a different distribution of *MED12* mutations according to the histology of uterine fibroids (7). Other studies have shown that mutations in *MED12* in tumors other than uterine fibroids and breast fibroadenomas were rare (22); suggesting that *MED12* is expressed differently depending on the pathology. Mutations also vary according to racial origin as new mutations were discovered in our patients compared to mutations found in others populations. This could be explained, as described above, by the fact that *MED12* exhibits variable expressivity following histology of the disease but also according to the race, which may be a determinant on racial prevalence distribution of uterine fibroids and breast fibroadenomas.

The differences between uterine fibroids and breast fibroadenomas were also confirmed by estimating the rate of evolution of mutations indicating that mutations evolve differently both at

intra and inter tumor levels. Indeed, the deletion in the intronic region seemed to evolve faster than all other mutations found in each of the diseases. The 1349G>A mutation, which moved more rapidly in cases of uterine fibroids than in cases of breast fibroadenomas (0.121 vs 0.027) further demonstrates that uterine fibroids and breast fibroadenomas could evolve differently among Senegalese women.

This different evolution between uterine fibroids and breast fibroadenomas was also reflected in the demo-genetic evolution. The mismatch graph shows a unimodale distribution for uterine fibroids signal to population growth. For fibroadenomas of the breast, the distribution was multimodal indicating a stable population.

Finally, our results showed for the first time the involvement of *MED12* in both diseases in Senegalese women with the presence of mutations only localized in the affected tissues. The fact that exon 2 is under positive selection, further confirms its involvement in the development of both uterine fibroids and breast fibroadenomas among Senegalese women. It would be interesting to determine the role of this region in the synthesis of MED12 protein in patients with uterine fibroids and breast fibroadenomas.

The present study also open avenues for understanding the mechanisms involved in racial variation in prevalence of uterine fibroids and breast fibroadenomas. The variable expressivity of the *MED12* according to the study population suggests its role in the distribution of uterine fibroids and breast fibroadenomas.

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Conflict of interest

The authors declared no conflict of interest.

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