

Implication of the Cytochrome *P-450c17α* (*CYP17α*) Gene in the Tumor Progression of Uterine Fibroids in Senegalese Women

Diallo Mariama¹, Ciss Daouda², Keneme Bineta¹, Gaye Alfousseyni², Sembene Pape Mbacke^{1,3}

¹Department of Animal Biology, Faculty of Science and Technology, University Cheikh Anta Diop of Dakar, Dakar, Senegal

²Maternity and Gynecology Obstetrics Service, Idrissa Pouye Hospital, Dakar, Senegal

³Biology Center for Population Management (GENGESPOP), Institute of Research and Development, Department of Animal Biology, Faculty of Science and Technology, University Cheikh Anta Diop of Dakar, IRD/Bel-Air, Dakar, Senegal

Email address:

mariama7.diallo@ucad.edu.sn (D. Mariama), drdaoudaciss@yahoo.fr (C. Daouda), bineta.keneme@ucad.edu.sn (K. Bineta), alfgaye@yahoo.fr (G. Alfousseyni), mbacke.sembene@ucad.edu.sn (S. P. Mbacke)

To cite this article:

Diallo Mariama, Ciss Daouda, Keneme Bineta, Gaye Alfousseyni, Sembene Pape Mbacke. Implication of the Cytochrome *P-450c17α* (*CYP17α*) Gene in the Tumor Progression of Uterine Fibroids in Senegalese Women. *International Journal of Genetics and Genomics*. Vol. 9, No. 4, 2021, pp. 67-77. doi: 10.11648/j.ijgg.20210904.12

Received: September 20, 2021; **Accepted:** October 14, 2021; **Published:** October 28, 2021

Abstract: Uterine fibroids, also known as leiomyomas, uterine myomas or fibroleiomyomas, are benign, encapsulated uterine tumors consisting of smooth muscle fibers of the uterus and connective tissue, the most common in the female genital tract. They affect 20 to 25% of women of childbearing age and are 3 to 9 times more common in black women than in white women, and nearly 70% of those close to 50 years of age. Uterine myomas are a real public health problem. The cost of treating them is expensive and the only treatment deemed effective is surgery. The rapid progression of leiomyomas during the childbearing years and regression after menopause indicate that estrogen plays a key role as a growth factor for these tumors. To contribute to the knowledge of the etiological factors involved in the tumor process of uterine fibroids, the study of the *Cytochrome P-450c17α* gene (*CYP17α*), involved in the hydroxylation of estrogen, has been carried out. Our study population consisted of 57 patients with uterine fibroids. After sampling (tumour tissue and peripheral blood), molecular analysis were done (DNA extraction, PCR-sequencing). Raw data were submitted to Mutation Surveyor 5.0.1 for mutation identification and AlamutVisual 2.12 software. The pathogenicity of each non-synonymous mutation was evaluated using Polyphen-2, Mutation Taster and SIFT. After cleaning, correction and alignment of sequences with BioEdit 7.0.8.0, genetic diversity, genetic differentiation and polymorphism of the gene *CYP17α* in correlation with epidemiological factors were determined with DNASP 5.10.01, MEGA 7.0.14, Arlequin 3.5.3.1 and the statistical software RStudio 3.5.1. Our results showed 84 mutations characterizing a high rate of tumor tissue polymorphism but also a genetic difference between tumor and peripheral blood. The mutation c.-34T>C which is located in the 5' promoter region at 34 bp upstream of the translation initiation site was found in patients with uterine fibroids. No genetic structuring of the *CYP17α* gene according to clinico-pathological parameters was observed. In conclusion, the cytochrome P450 enzymes responsible for highly specific reactions in the steroid biosynthesis pathway are gaining interest as molecular targets, given their role clé in the formation of various very potent endogenous steroid hormones. Indeed, current treatments for tumors, particularly fibroids, are mainly surgical and expensive. It is therefore essential to develop and evaluate alternatives to surgical procedures.

Keywords: Leiomyoma Uterine, *Cytochrome P-450c17α* (*CYP17α*), Senegalese Patients

1. Introduction

Uterine fibroids, also known as leiomyomas, uterine myomas or fibroleiomyomas, are benign, encapsulated

uterine tumors composed of smooth muscle fibers of the uterus and connective tissue, the most common in the female genital tract. They can be single or multiple, of variable size and location [1]. They affect 20-25% of women of

reproductive age and are 3-9 times more common in black women than white women and nearly 70% of those near 50 years of age [2-5].

Uterine leiomyoma although representing a benign pathology often poses serious problems, causing significant morbidity and deterioration of quality of life [6]. Its medical management, the social impact and cost of the intervention, the possible complications, the work stoppage it causes, but also the psychological impact [7], impose a research on the determinism of this frequent lesion in populations of black women living in an underdeveloped context. In Senegal, studies conducted Sall [8] indicated that surgeries performed for the treatment of uterine myomatosis were 37.3% of all gynecological surgical procedures performed at the Centre Hospitalier National (CHN) de Pikine over a 5-year (60 months) period from January 2010 to December 2014.

In addition, evidence is focused on the study of molecular mechanisms of disease development and the influence of ethnicity. Ovarian steroids play an important role in the development of leiomyoma. The rapid progression of leiomyomas during the reproductive years and regression after menopause indicates that estrogen plays a key role as a growth factor for these tumors [9]. At the molecular level, independent studies have suggested the involvement of certain genes in the occurrence and even progression of these hormone-dependent tumors [10-11]. And among the genes studied, the polymorphism of the *Cytochrome P-450c17α* (*CYP17α*) gene, involved in estrogen hydroxylation, may have an influence on the degree of estrogen metabolism. Nevertheless, despite all the efforts made in fibroid research, the exact causes of tumor initiation and/or promotion remain incompletely elucidated. Furthermore, no clear indication on the racial disparity in the distribution of uterine fibroids has yet been obtained in Africa or in the rest of the world. This points to a great need to undertake a study on the genetic determinants of uterine fibroids in a black population, in particular in Senegal.

In this article we will contribute to the knowledge of the etiological factors involved in the tumoral process of uterine fibroids, which will allow us to identify and characterize the mutations of the *CYP17α* gene in uterine fibroids that could be determinant, then to compare the mutational penetrance of this *CYP17α* gene in patients with controls. Finally, to determine the evolutionary process of the genetic alterations according to the clinico-pathological parameters of the patients.

2. Materials and Methods

2.1. Samples Collection

The data consisted of 57 patients with uterine fibroids scheduled for surgery at the Maternity and Obstetrics Department of the Hôpital Général Idrissa Pouye. Prior to any inclusion of the cohorts, each patient recruited was interviewed. The objectives of the study, the protocol, the benefits, the confidentiality and their rights were explained to

them to give them the opportunity to accept or refuse to participate. In the case of acceptance, a duly completed and signed informed consent form was required for admission to the study. An epidemiological survey form was made available to the patients to collect information about their identity (patient age, ethnicity, height, weight, number of pregnancy, number of childbirth), their lifestyle (physical activity, diet, contraception).

After sampling, the samples were sent directly to the laboratory where the blood tissues (18 in number to serve as controls) were preserved in EDTA tubes, whereas the tumor tissues (57) were preserved in 96% alcohol in a refrigerator at -20°C for the various molecular analysis. According to the rules set forth by the National Committee of Ethics for Health Research (CNERS) of Senegal and in accordance with the procedures established by the Cheikh Anta Diop University of Dakar (UCAD) for all research involving human participants, ethical approval was obtained for this study.

Table 1. Characteristics of epidemiological parameters.

Epidemiological parameters	Groups	Number of patients (%)
Age (n= 57)	≤35	20 (35.57%)
	>35 ≤45	25 (43.86%)
	>45	12 (21.05%)
Parity (n= 57)	Nulliparous	43 (75.44%)
	Multiparous	14 (24.56%)
Gestivity (n= 57)	Nulligeste	31 (54.38%)
	Multigeste	26 (45.61%)
Age at menarche (n= 57)	[12-14]	30 (52.63%)
	>14	27 (47.37%)
Diet (n= 57)	Meat preference	22 (38.6%)
	Vegetarian	13 (22.80%)
	Mixed	22 (38.6%)
Marital status (n= 57)	Married	34 (59.65%)
	Single	19 (33.33%)
	Divorced	4 (7.02%)
	Wolof	19 (33.33%)
Ethnicity (n= 57)	Poulaar	15 (26.31%)
	Serere	7 (12.28%)
	Diola	11 (19.3%)
	Lebou	5 (8.77%)
	Insufficiency	6 (10.52%)
BMI (n=57)	Normal	32 (56.14%)
	Obesity	19 (33.33%)

2.2. Dna Extraction and Pcr-sequencing of *CYP17α* Gene

Total DNA was extracted from tumour tissue and blood using the DNasy blood and tissue kit (Qiagen). Amplification was performed in a 50 µl reaction volume with primers 5'-TCCTGAGCCCAGATACCAT-3' and 5'-CCGCCAGAGAAGTCCT-3 flanking the 5'UTR region at exon 1 and is 612 bp. Electrophoretic migration on 1.5% agarose gel was performed to confirm the amplification. Sequencing reactions were performed in a MJ Research PTC-224 Peltier thermal cycler with ABIPRISM BigDye TM Terminator Cycle kits. Each sample was sequenced using forward primer. Fluorescent fragments were purified using the BigDye Xterminator purification protocol. Samples were

suspended in distilled water and subjected to electrophoresis in ABI 3730xl sequencer (Applied Biosystems).

2.3. Molecular Analysis

2.3.1. Analysis of Chromatograms

The sequences obtained were submitted to the Mutation Surveyor software version 5.0.1 (www.softgenetics.com). This software compares the submitted chromatograms with the reference sequence of the gene of interest incorporated in the database of this software but also with sequences from the Genbank database under the accession number NT_030059_104589788.

2.3.2. Pathogenicity of Identified Mutations

The mutations thus detected are then submitted to the SIFT, Mutation Taster and Polyphen 2 databases, which are software programs that help interpret genetic mutations identified in the human genome. These programs use scores to predict whether an amino acid substitution will affect the function of the protein and, therefore, potentially alter the phenotype.

2.3.3. Quantification of the Mutational Penetrance of the Gene

After correction, the raw sequences were carefully aligned with BioEdit 7.0.8.0 [12] using the Clustal-W algorithm [13].

The genetic diversity parameters allow the total length of sites without gaps, sample size, number of invariant and variable sites, total number of mutations, number of haplotypes, average number of nucleotide differences between sequences, nucleotide frequency, nature of mutations, and mutation rate to be identified. These parameters were determined in each group (control and tumor tissue) with DnaSP version 5.10 [14] and MEGA version 7.0.14 [15]. On the other hand we also have the genetic diversity indices that include haplotypic diversity and nucleotide diversity that were brought out with DnaSP software version 5.10 [14] to apprehend the evolution of genetic mutations.

Comparison of amino acid frequencies between control

and uterine myoma tissues was performed using MEGA software version 7.0.14 [15]. The calculation of amino acid frequencies was done using the 1st nucleotide starting exon 1 of the gene and choosing the 1st reading frame. To see if there is a significant difference on the frequency distribution of each amino acid between blood and tumor tissue, the database was submitted to R software version 3.3.1 [16]. The Shapiro Wilk normality test was performed to see if the data follow a normal distribution. In the case of a normal distribution, Student's t-test is performed for comparison of means; otherwise the Wilcox test is used. A significance level of 5% was used.

2.3.4. CYP17 α Gene Polymorphism and Epidemiological Factors

The genetic structuring according to the epidemiological parameters gesity (number of pregnancies), parity (number of childbirth), early menarche, age of the patient, ethnicity and BMI (Body Mass Index) was also apprehended by carrying out an analysis of molecular variance (AMOVA: Analysis of Molecular Variance) with the Arlequin 3.5.1.3 software [17] with the aim of seeing which are the risk factors involved in this pathology in Senegal.

3. Results and Discussion

3.1. Results

3.1.1. Nature and Position of Mutations

In patients with uterine myomas, 84 mutations were found, of which 68 (80.95%) were newly identified and 16 (19.06%) were already found in the databases. The c.-34T>C mutation located in the 5' promoter region 34 bp upstream of the translation initiation site was found in patients with uterine fibroids. Non-synonymous mutations (42) are in the majority compared to synonymous mutations (16). Mutations inducing the loss of the methionine initiator codon were also found (c.2T>A p.Met1Lys; c.3G>A p.Met1Ile), two nonsense codons (c.5G>A p.Trp2*; c.81C>A p.Tyr27*) were also identified in some patients (Table 2).

Table 2. Nature and position of CYP17 α gene mutations.

Position of mutations	dbSNP	Effect on coding	Amino acid affected
c.-93G>T	New	No effect on the splice site	
c.-86C>T	New	No effect on the splice site	
c.-77G>C	New	No effect on the splice site	
c.-75C>G	New	No effect on the splice site	
c.-73T>A	New	No effect on the splice site	
c.-49G>T	New	No effect on the splice site	
c.-46C>A	New	No effect on the splice site	
c.-43C>A	New	No effect on the splice site	
c.-40C>A	New	No effect on the splice site	
c.-38C>A	New	No effect on the splice site	
c.-34T>C	rs743572	Creation of a CCACC box site	
c.-31T>C	rs1237281550	No effect on the splice site	
c.-30G>C	New	No effect on the splice site	
c.-28C>T	New	No effect on the splice site	
c.-24G>C	New	No effect on the splice site	
c.-21G> T	New	No effect on the splice site	
c.-17G>C	New	No effect on the splice site	

Position of mutations	dbSNP	Effect on coding	Amino acid affected
c.-15C>T	rs140012815	Cryptic site weakly activated	
c.-5C>A	New	Strongly activated cryptic site	
c.2T>A	New	Loss of the Met initiator codon	p.Met1Lys
c.3G>A	rs61754262	Loss of the Met initiator codon	p.Met1Ile
c.5G>A	New	Non-sens	p.Trp2*
c.12C>T	rs565323692	Synonymous	p.Leu4Leu
c.15G>T	New	Synonymous	p.Val5Val
c.32C>T	rs72559703	Non-Synonymous	p.Thr11Ile
c.38C>A	New	Non-synonymous/ activated acceptor	p.Ala13Asp
c.62G>A	rs61754263	Non-Synonymous	p.Arg21Lys
c.66C>G	rs762563	Non-Synonymous	p.Cys22Trp
c.70G>T	New	Non-Synonymous	p.Gly24Cys
c.73G>C	New	Non-Synonymous	p.Ala25Pro
c.79T>C	New	Non-Synonymous	p.Tyr27His
c.80A>C	rs757251521	Non-Synonymous	p.Tyr27Ser
c.81C>A	rs104894152	Non-sens	p.Tyr27*
c.86A>C	New	Non-Synonymous	p.Lys29Thr
c.87G>A	New	Synonymous	p.Lys29Lys
c.88A>G	New	Non-Synonymous	p.Ser30Gly
c.89G>A	rs1341416067	Non-Synonymous	p.Ser30Asn
c.90C>A	New	Non-Synonymous	p.Ser30Arg
c.91C>G	New	Non-Synonymous	p.Leu31Val
c.94C>A	New	Non-Synonymous	p.Leu32Met
c.96G>C	rs1398304295	Non-Synonymous	p.Leu32Leu
c.98C>A	New	Non-Synonymous	p.Ser33Tyr
c.99C>T	rs939625154	Synonymous	p.Ser33Ser
c.100C>G	New	Non-Synonymous	p.Leu34Val
c.102C>G	New	Synonymous	p.Leu34Leu
c.106C>G	New	Non-Synonymous	p.Leu36Val
c.108G>C	New	Synonymous / Activated acceptor site	p.Leu36Leu
c.109G>T	New	Non-Synonymous	p.Val37Leu
c.111G>A	New	Creation of a splicing site	p.Val37Val
c.112G>A	New	Non-Synonymous	p.Gly38Ser
c.112G>T	New	Non-Synonymous	p.Gly38Cys
c.120G>C	New	Synonymous	p.Leu40Leu
c.121C>G	New	Non-Synonymous	p.Pro41Ala
c.129C>G	New	Synonymous	p.Leu43Leu
c.134G>A	New	Non-Synonymous	p.Arg45Lys
c.137A>G	New	Non-Synonymous	p.His46Arg
c.138C>A	New	Non-Synonymous	p.His46Gln
c.138C>T	rs6162	Synonymous	p.His46His
c.141C>A	New	Synonymous	p.Gly47Gly
c.142C>T	New	Non-Synonymous	p.His48Tyr
c.144T>A	New	Non-Synonymous	p.His48Gln
c.147G>A	New	Non-Synonymous	p.Met49Ile
c.154A>T	New	Non-Synonymous	p.Asn52Tyr
c.156C>A	New	Non-Synonymous	p.Asn52Lys
c.159C>T	New	Synonymous	p.Phe53Phe
c.164A>T	New	Non-Synonymous	p.Lys55Met
c.167T>A	New	Non-Synonymous	p.Leu56Gln
c.171G>T	New	Non-Synonymous	p.Gln57His
c.179A>G	New	Non-Synonymous	p.Tyr60Cys
c.180T>G	New	Non-sens	p.Tyr60*
c.183C>G	New	Synonymous	p.Gly61Gly
c.188T>G	New	Non-Synonymous	p.Ile63Ser
c.189C>G	New	Non-Synonymous	p.Ile63Met
c.194C>A	New	Synonymous	p.Ser65Ser
c.195G>C	New	Synonymous	p.Ser65Ser
c.195G>T	rs6163	Synonymous	p.Ser65Ser
c.197T>A	New	Non-Synonymous	p.Val66Asp
c.199C>A	New	Non-Synonymous	p.Arg67Ser
c.200G>A	rs376074317	Non-Synonymous	p.Arg67His
c.213G>A	New	Synonymous	p.Lys71Lys
c.215C>A	New	Non-Synonymous	p.Thr72Asn
c.240G>T	New	Non-Synonymous	p.Gln80His
c.245C>A	New	Non-Synonymous	p.Ala82Asp
c.267G>C	New	Non-Synonymous	p.Lys89Asn

Most of the non-synonymous mutations are considered as neutral polymorphic but 20 variants (47.61%) are considered as potentially pathogenic by Mutation Taster, 12 (28.57%) mutations are considered as deleterious by SIFT. On the other hand, most of the mutations are considered as potentially harmful by polyphen-2. The mutations (c.91C>G p.Leu31Val;

c.112G>A p.Gly38Ser; c.112G>T p.Gly38Cys; c.164A>T p.Lys55Met; c.167T>A p.Leu56Gln; c.179A>G p.Tyr60Cys; c.189C>G p.Ile63Met; c.197T>A p.Val66Asp; c.245C>A p.Ala82Asp) are considered likely pathogenic, deleterious, and damaging by these 3 prediction software (Table 3).

Table 3. Pathogenicity of *CYP17A* gene mutations.

Mutations	Amino acids	Effect on coding	Mutation Taster (score)	SIFT (score)	Polyphen-2 (score)
c.2T>A	p.Met1Lys	Loss of the Met initiator codon			
c.3G>A	p.Met1Ile	Loss of the Met initiator codon			
c.32C>T	p.Thr11Ile	Non-Synonymous	Polymorphism (1)	Tolerated (0.63)	Benign (0.001)
c.38C>A	p.Ala13Asp	Non-Synonymous	Polymorphism (1)	Deleterious (0.02)	Possible damage (0.91)
c.62G>A	p.Arg21Lys	Non-Synonymous	Polymorphism (1)	Tolerated (1)	Benign (0.001)
c.66C>G	p.Cys22Trp	Non-Synonymous	Polymorphism (1)	Tolerated (0.18)	Possible damage (0.91)
c.70G>T	p.Gly24Cys	Non-Synonymous	Polymorphism (1)	Deleterious (0.01)	Possible damage (0.998)
c.73G>C	p.Ala25Pro	Non-Synonymous	Polymorphism (0.847)	Tolerated (0.2)	Benign (0.028)
c.79T>C	p.Tyr27His	Non-Synonymous	Polymorphism (0.968)	Tolerated (0.54)	Possible damage (0.989)
c.80A>C	p.Tyr27Ser	Non-Synonymous	Polymorphism (0.897)	Tolerated (0.41)	Benign (0.221)
c.86A>C	p.Lys29Thr	Non-Synonymous	Polymorphism (0.994)	Tolerated (0.2)	Benign (0.051)
c.88A>G	p.Ser30Gly	Non-Synonymous	Pathogen (1)	Tolerated (0.22)	Dommmageable (0.998)
c.89G>A	p.Ser30Asn	Non-Synonymous	Pathogen (1)	Tolerated (0.18)	Dommmageable (1)
c.90C>A	p.Ser30Arg	Non-Synonymous	Pathogen (1)	Tolerated (0.2)	Dommmageable (1)
c.91C>G	p.Leu31Val	Non-Synonymous	Pathogen (1)	Deleterious (0.05)	Dommmageable (0.992)
c.94C>A	p.Leu32Met	Non-Synonymous	Pathogen (0.825)	Tolerated (0.12)	Dommmageable (0.975)
c.98C>A	p.Ser33Tyr	Non-Synonymous	Polymorphism	Tolerated (0.37)	Benign (0.006)
c.100C>G	p.Leu34Val	Non-Synonymous	Pathogen (1)	Tolerated (0.15)	Dommmageable (0.991)
c.106C>G	p.Leu36Val	Non-Synonymous	Pathogen (0.758)	Tolerated (0.11)	Benign (0.005)
c.109G>T	p.Val37Leu	Non-Synonymous	Pathogen (0.995)	Tolerated (0.07)	Benign (0.104)
c.112G>A	p.Gly38Ser	Non-Synonymous	Pathogen (1)	Deleterious (0)	Possible damage (1)
c.112G>T	p.Gly38Cys	Non-Synonymous	Pathogen (1)	Deleterious (0)	Possible damage (1)
c.121C>G	p.Pro41Ala	Non-Synonymous	Pathogen (0.582)	Tolerated (0.21)	Possible damage (0.977)
c.134G>A	p.Arg45Lys	Non-Synonymous	Polymorphism (1)	Tolerated (0.06)	Benign (0.012)
c.137A>G	p.His46Arg	Non-Synonymous	Polymorphism (1)	Tolerated (0.65)	Benign (0.007)
c.138C>A	p.His46Gln	Non-Synonymous	Polymorphism (1)	Tolerated (0.66)	Possible damage (0.853)
c.142C>T	p.His48Tyr	Non-Synonymous	Polymorphism (1)	Tolerated (1)	Possible damage (0.991)
c.144T>A	p.His48Gln	Non-Synonymous	Polymorphism (1)	Tolerated (0.65)	Benign (0.028)
c.147G>A	p.Met49Ile	Non-Synonymous	Polymorphism (1)	Tolerated (0.37)	Benign (0.376)
c.154A>T	p.Asn52Tyr	Non-Synonymous	Polymorphism (1)	Tolerated (0.4)	Benign (0.037)
c.156C>A	p.Asn52Lys	Non-Synonymous	Polymorphism (1)	Tolerated (0.94)	Possible damage
c.164A>T	p.Lys55Met	Non-Synonymous	Pathogen (0.998)	Deleterious (0.03)	Possible damage
c.167T>A	p.Leu56Gln	Non-Synonymous	Pathogen (1)	Deleterious (0.01)	Possible damage (1)
c.171G>T	p.Gln57His	Non-Synonymous	Pathogen (1)	Tolerated (0.11)	Possible damage (1)
c.179A>G	p.Tyr60Cys	Non-Synonymous	Pathogen (1)	Deleterious (0.06)	Possible damage (1)
c.188T>G	p.Ile63Ser	Non-Synonymous	Polymorphism (0.818)	Deleterious (0.01)	Possible damage (0.974)
c.189C>G	p.Ile63Met	Non-Synonymous	Pathogen (0.799)	Deleterious (0.04)	Possible damage (0.996)
c.197T>A	p.Val66Asp	Non-Synonymous	Pathogen (1)	Deleterious (0)	Possible damage (0.507)
c.199C>A	p.Arg67Ser	Non-Synonymous	Polymorphism (1)	Tolerated (0.4)	Possible damage (0.997)
c.200G>A	p.Arg67His	Non-Synonymous	Polymorphism (1)	Tolerated (0.23)	Possible damage (0.951)
c.215C>A	p.Thr72Asn	Non-Synonymous	Polymorphism (1)	Tolerated (0.31)	Possible damage (0.464)
c.240G>T	p.Gln80His	Non-Synonymous	Pathogen (0.78)	Tolerated (0.1)	Benign (0.339)
c.245C>A	p.Ala82Asp	Non-Synonymous	Pathogen (1)	Deleterious (0.02)	Possible damage (1)
c.267G>C	p.Lys89Asn	Non-Synonymous	Pathogen (1)	Tolerated (0.46)	Possible damage (0.995)

3.1.2. Mutational Penetrance

(i). Genetic Diversity of the *CYP17A* Gene

Analysis of the 612 sites obtained after alignment of the dataset in the 57 patients with uterine fibroids shows 133 polymorphic sites including 74 informative sites with a total number of mutations of 162 and an average number of nucleotide differences of 27.959. The nature of the mutations indicates a transition/transversion ratio of 0.681

showing that transversions (59.1%) are more numerous than transitions (40.1%). The rate of synonymous substitutions (mutations that do not lead to amino acid changes) and non-synonymous substitutions (mutations that lead to amino acid changes) indicates that non-synonymous substitutions (dN=0.038) are lower than synonymous substitutions (dS=0.061). In contrast, only one (1) polymorphic site is noted at the control level (18). These results are presented in Table 4.

(ii). Amino acid Frequency of Exon 1 of the *CYP17α* Gene

The results show a significant decrease in the frequency of some amino-acid in tumor tissues compared to controls such

as: Histidine, Lysine, Leucine and Methionine, on the other hand the level of Phenylalanine increased. These results are presented in Table 5.

Table 4. Genetic diversity parameters of the *CYP17α* gene.

Parameters	Controls	affected
Sample sizes	18	57
Total number of sites	612	612
Number of polymorphic sites	1	133
invariable Sites	611	479
Informative sites	1	74
Variable site	0	59
Total number of mutations Eta	1	162
Number of Haplotypes Ha	2	24
Average number of nucleotide differences k	0.503	27.959
Transition (%)		40.9%
Transversion (%)		59.1%
Nucleotide frequencies	A: 20.83%	A: 23.86%
	T/U: 21.66%	T/U: 21.28%
	C: 32.84%	C: 30.43%
	G: 24.66%	G: 24.44%
Mutation rate R		0.681
Synonymous substitutions (dS)		0.061±0.013
Non-synonymous substitution (dN)		0.038±0.007
Z-test Selection (dN<dS)		Prob=0.025; stat=1.974
Haplotypes diversity Hd		0.850±0.042
Nucleotide diversity Pi		0.04569±0.00268

Table 5. Frequency of amino acids and their *p*-value in controls and uterine fibroids.

Amino acids	control	uterine fibroid	P-value
Ala	3.555	4.364	0.3705
Cys	1.185	1.288	0.6887
Asp	0	0.062	0.3328
Glu	1.185	1.621	0.2086
Phe	4.740	5.403	0.0300*
Gly	7.110	7.024	0.5821
His	5.925	5.258	<0.001***
Ile	2.370	3.283	0.1927
Lys	8.295	7.544	0.0043**
Leu	17.775	15.253	<0.001***
Met	3.555	3.221	<0.001***
Asn	3.555	3.554	0.7559
Pro	9.480	9.622	0.6565
Gln	2.370	2.992	0.6732
Arg	5.925	5.923	0.673
Ser	4.740	4.697	0.558
Thr	4.740	4.759	0.9662
Val	7.110	7.294	0.6518
Trp	2,370	2.369	0.7795
Tyr	4.016	4.468	0.0753

Significance codes: $p < 0.001$ '***' $p \approx 0.001$ '**' $p \approx 0.01$ '*'

3.1.3. Genetic Polymorphism and Epidemiological Factors

The analysis of genetic differentiation reveals that there is no genetic structuring according to the epidemiological parameters because the value of the *p*-values is higher than 0.05 and therefore not significant (Table 6 and Table 7).

Table 6. Genetic differentiation by epidemiological factors.

Clinical parameters	Genetic differentiation factor Fst		
Groups Sub-groups	Intra sub-group	Inter sub-groups	
Age	Fst	Between sub-groups	Fst (<i>p</i> -value)
≤35	-0.03842	≤35 & >35 ≤45	-0.03585 (0.93694)
>35 ≤45	-0.03973	≤35 & >45	-0.04295 (0.67568)
>45	-0.04952	>35 ≤45 & >45	-0.04959 (0.89189)
Parity	Fst	Between sub-groups	Fst (<i>p</i> -value)

Clinical parameters		Genetic differentiation factor Fst	
Groups	Sub-groups	Intra sub-group	Inter sub-groups
Age	Fst	Between sub-groups	Fst (p-value)
Nulliparous	0.07378	Nullipare & Multipare	0.07516 (0.05405)
Multiparous	0.07938		
Gestity	Fst	Between sub-groups	Fst (p-value)
Nulligeste	0.02559	Nulligeste & Multigeste	0.02579 (0.10811)
Multigeste	0.02602		
Age at menarche	Fst	Between sub-groups	Fst (p-value)
[12-14]	-0.02015	[12-14] & >14	-0.02115 (0.63964)
>14	-0.02226		
Diet	Fst	Between sub-groups	Fst (p-value)
Meat preference	-0.00573	Meat & Vegetarian	-0.00987 (0.34234)
Vegetarian	-0.00638	Meat & Mixed	-0.02887 (0.82883)
Mixed	-0.00846	Vegetarian & Mixed	0.02999 (0.21622)
Marital status	Fst	Between sub-groups	Fst (p-value)
Married	-0.04173	Married & Single	-0.02973 (0.86486)
Single	-0.03262	Married & Divorced	-0.07287 (0.57658)
Divorced	-0.02872	Single & Divorced	-0.04680 (0.33333)
Ethnicity	Fst	Between sub-groups	Fst (p-value)
Wolof	0.05124	Wolof & Poular	0.07187 (0.15315)
Poular	0.07083	Wolof & Sérère	-0.06014 (0.65766)
Serere	0.06823	Wolof & Diola	-0.04241 (0.68468)
Diola	0.05991	Wolof & Lébou	0.09908 (0.15315)
Lebou	0.11029	Poular & Sérère	-0.04936 (0.54054)
		Poular & Diola	0.11832 (0.04505)
		Poular & Lébou	0.40922 (0.00000)
		Sérère & Diola	-0.02471 (0.36937)
		Sérère & Lébou	0.26003 (0.00901)
		Diola & Lébou	0.04077 (0.36036)
BMI	Fst	Between sub-groups	Fst (p-value)
Insufficiency	-0.02863	Insufficiency & Normal	-0.03597 (0.66667)
Normal	-0.03413	Insufficiency & Obesity	-0.09388 (0.81982)
Obesity	-0.02534	Normal & Obesity	-0.01834 (0.43243)

Table 7. Genetic differentiation of the general population according to clinical parameters.

Epidemiological parameters	Percentage of variation (%)		Fst (p-value)
	Intra-group	Inter-groups	
Age	104.13	-4.13	-0.04133 (0.96676)
Parity	92.48	7.52	0.07516 (0.07820)
Gestity	97.42	2.58	0.02579 (0.14370)
Age at menarche	102.12	-2.12	-0.02115 (0.71359)
Diet	100.69	-0.69	-0.00693 (0.42424)
Marital status	103.77	-3.77	-0.03769 (0.75660)
Ethnicity	93.47	6.53	0.06534 (0.09580)
BMI	103.06	-3.06	-0.03062 (0.70772)

3.2. Discussion

This study included 57 patients with uterine myomas. The objective was to identify mutations in the *CYP17α* gene (nuclear gene) involved in the development of uterine fibroids, to determine the mutational penetrance as well as the clinico-pathological parameters involved in the development of this disease in the study population.

Analysis of nucleotide variability identified several mutations in the *CYP17α* gene in fibrotic tissues (68.1) and a single mutation (1) in controls. This indicates that these variations could be involved in the development of uterine fibroids in our patients. The fact that some patients with uterine fibroids do not show alterations indicates that the *CYP17α* gene is not the only gene involved in fibroids. Indeed, other genes have been incriminated in the occurrence

of uterine fibroids, the most important of which is the *MED12* gene [18, 19].

The human cytochrome *P450-17A1* (*CYP17A1*) enzyme acts at a key time point in human steroidogenesis, controlling levels of mineralocorticoids influencing blood pressure, glucocorticoids involved in immune and stress responses, and androgens involved in development and reproductive tissue homeostasis [20]. The *CYP17A1* enzyme catalyzes both 17α-hydroxylase and 17,20-lyase activities and also has a modest degree of 16α-hydroxylase activity [21]. Steroid 17α-hydroxylase (steroid 17α monooxygenase) converts pregnenolone to 17-hydroxypregnenolone and converts progesterone to 17-hydroxyprogesterone, a precursor or soft synthesis of testosterone and estrogen. Although steroid 17α-hydroxylase and 17,20-lyase activities can be readily distinguished, examination of circulating venous steroid

products have shown that both activities reside in a single protein, *CYP17A1* encoded by the *CYP17 α* gene [22; 23]. Thus, the *CYP17A1* enzyme is a key branch point in human steroid hormone synthesis.

In women, *CYP17 α* is expressed in the adrenal glands, adipose tissue, thecal cells of the ovary, and corpus luteum [24-26]. The *CYP17A1* enzyme converts 21-carbon steroids to 19-carbon androgens in two chemical transformations [27]. First, pregnenolone or 21-carbon progesterone is hydroxylated. The 17 α hydroxypregnenolone product can undergo a second 17,20-lyase reaction in the same active site to yield 19-carbon dehydroepiandrosterone (DHEA), the androgen precursor for all sex steroids. Thus, mutations or clinical inhibition affecting both reactions of *CYP17A1* block the production of androgens and glucocorticoids. Glucocorticoid deficiency results in the secretion of adrenocorticotropin hormone, which leads to overproduction of mineralocorticoids [28].

Mutations in the promoter region of *CYP17 α* are very common in patients with uterine fibroids. These mutations could induce overexpression of *CYP17A1* enzyme synthesis. Indeed, the work of [29] showed that *CYP17A1* expression in thecal cells isolated from women with polycystic ovary syndrome is consistently elevated compared to normal cells. To investigate the mechanism of the increased accumulation of *CYP17A1* mRNA in ovarian tumor cells, [29] examined the activities of the *CYP17 α* promoter and steroidogenic acute regulatory protein (StAR). Basal *CYP17 α* promoter activity was 4-fold higher in ovarian tumor cells than in theca cells isolated from normal ovaries. The authors concluded from these data that basal and cAMP-dependent *CYP17 α* gene transcription is increased in tumor cells.

In addition, the c.-34T>C mutation creates a new CCACC box site and thus an additional promoter. [30] first identified this SNP and hypothesized that the C allele might upregulate gene expression by primarily increasing serum hormones including androstenedione and estradiol (E2). This polymorphism is common: the CC genotype is present in 11-19% of white North American women and 6-16% of African American women [31]. Several studies have hypothesized that the C allele of *CYP17 α* may be a marker of increased steroidogenesis [32; 33]. This SNP was then investigated in uterine fibroids in populations with high ethnic diversity such as South Africa, Brazil, and the Caribbean [34-36]. In South Africa, Amant *et al.* [34] reported a strong association between uterine fibroids and the presence of the C mutant allele. The work of [36], based on the allelic distribution of *CYP17 α* showed a predominance of the homozygous TT genotype with a frequency of 52% followed by the heterozygous TC genotype with a frequency of 41%; the CC genotype constituting only 6% of cases. In contrast, in Brazil, no association was noted between uterine fibroids and the presence of the C mutant allele [35].

The c.3G>A mutation alters the 1st amino acid of exon 1 of the *CYP17 α* gene, namely methionine, and thus leads to the loss of the translation initiator codon. This loss could result in an inhibition of the synthesis of the *CYP17A1*

enzyme and therefore a blockage of 17 α -hydroxylase and 17,20-lyase activities in patients with this polymorphism. This is further confirmed by the discovery of the c.5G>A variant. This variant corresponds to a premature stop codon at Tryptophan in codon 2 (p.Trp2) and induces the genetic disorder of *CYP17A1* that results in 17 α -hydroxylase/17,20-lyase deficiency. The mutation at position 17 could induce loss of C17 cleavage of *CYP17A1* enzyme and thus overexpression of sex steroids by inhibition of regulatory metabolism.

The c.81C>A and c.180T>G mutations, which correspond respectively to a replacement of Tyrosine 27 by a stop codon (p.Tyr27*) and (p.Tyr60*) are also pathogenic variants found in uterine fibroids. This polymorphism which creates a truncated enzyme is also found in other pathologies. Indeed, studies by [37] indicated the presence of this variant in a 20 year old Turkish patient who presented with primary amenorrhea and sexual infantilism. The patient's steroid metabolism showed increased levels of mineralocorticoid precursors and low or undetectable plasma concentrations of 17 α -hydroxycorticoids, androgens and estrogens. The premise is that in this patient, both copies of the 17 α -hydroxylase gene are defective in the form of a homozygous stop codon in exon 1, significantly truncating the protein at the amino acid. The heme binding site, substrate binding pocket, and redox partner site, all of which have been reported to be essential for C17 catalytic activity, are missing. Therefore, *CYP17A1* will have no activity either as a 17 α -hydroxylase or as a 17,20-lyase.

The results are in agreement with the data in the literature. The mutations lead to a blockage of enzymatic synthesis in fibroids. This could be explained by an inhibition of the progesterone regulatory metabolism by the *CYP17A1* enzyme and thus an overproduction of the latter in patients. This suggests that the overproduction of progesterone is indeed essential for fibroid growth and that estrogen has an indirect effect. The hormonal dependence of uterine fibroid growth has long been accepted, and 17 β -estradiol (E2) has been considered the mitogen of uterine fibroids for decades [38]. However, using the xenograft model, [39] establish that progesterone (P4) is actually the driver of uterine fibroid growth. Although E2 itself is not a mitogen, it plays an essential role in their growth by sensitizing uterine fibroid cells to P4 through the upregulation of the progesterone receptor.

The involvement of the *CYP17 α* gene in the progression of uterine fibroids is further confirmed by evidence of genetic diversity. Among the variations found, transitional type mutations (40.9%) are lower than transversions (59.1%). The latter are at the origin of changes in the conformation of the protein structure, most often at the second codon position.

Analysis of amino acid composition showed an increase in phenylalanine and a decrease in amino acids such as histidine, lysine, leucine and methionine in tumor tissue. Histidine status appears to be important in skin dysfunction and various skin diseases, histidine deficiency, as in other essential amino acid (Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr,

Trp, Val, Arg, or Gln) significantly reduced hyaluronan levels in human skin fibroblasts [40]. Hyaluronan plays an important role in tissue repair and cell proliferation and migration in the skin [41]. Leucine is an essential amino acid for protein synthesis. Initiation of mRNA translation is the primary mechanism by which leucine stimulates protein synthesis. Additionally, similar to other amino acids, the carbon backbone of leucine can be used to generate ATP. However, leucine can also regulate several cellular processes such as protein synthesis, tissue regeneration and metabolism [42]. Methionine is no longer only for protein initiation, the redox cycle allows methionine residues to provide a catalytically effective antioxidant defense by reacting with oxidant species. The cycle also constitutes a reversible covalent post-translational modification analogous to phosphorylation. As with phosphorylation, enzymatically mediated oxidation and reduction of specific methionine residues functions as a regulatory process in the cell [43].

Although uterine fibroids are multifactorial tumors, the search for genetic contributors to epidemiological factors is relevant to the understanding of etiological factors involved in their developmental physiology. There is no structuring of the *CYP17a* gene according to epidemiological parameters. The *CYP17a* gene is only involved in tumor progression but not in tumor initiation. The development of uterine fibroids is associated with exposure to ovarian sex steroids. Estrogen can exert its mitogenic effect *via* estrogen-dependent growth factors. Growth factors are multifunctional cytokines that regulate several biological functions ranging from growth to differentiation to apoptotic cells. Growth factors are also associated with cell proliferation and differentiation, angiogenesis, extracellular matrix changes, and immunomodulation [44].

Despite ample clinical evidence that hormonal status is associated with fibroid development [44; 45], the precise functional roles of estrogen and progesterin in fibroid biology are still incompletely understood. For example, proliferation of human uterine smooth muscle-derived cells and uterine fibroid-derived cells is stimulated in a dose-dependent manner by 17 β -estradiol [46-48]. These results suggest that estrogens exert their pro-mitogenic effect through transcriptional mechanisms. There are conflicting experimental and clinical findings regarding a possible stimulatory, inhibitory, or facilitative role of progesterone in uterine fibroid development [49]. Progesterone appears to be essential for the growth of human fibroid xenografts, which is both counterintuitive, given the propensity of pregnancy to attenuate leiomyoma development and/or growth, and to enhance the tumor burden observed with antiprogesterins [50]. These conflicting findings, coupled with the fact that multiple fibroids are clonal and may have different growth characteristics in the same uterus, suggest that local and cellular mechanisms, possibly playing a role in their microenvironment, is a key factor in fibroid development and growth [51].

4. Conclusion

Fibroids affect millions of women worldwide and occur, in 60% of cases, in women of childbearing age, which constitutes a real public health problem. The prevalence of fibroids varies according to ethnicity, with women of African origin being at greater risk. Since these ethnic disparities cannot be fully explained by socioeconomic or environmental factors, the search for genetic contributors seems essential. Results on the genetic determinism of the *CYP17* gene showed a significant difference between controls and uterine myoma tissues.

Cytochrome P450 enzymes responsible for highly specific reactions in the steroid biosynthetic pathway are gaining interest as molecular targets, given their key role in the formation of various highly potent endogenous steroid hormones. Indeed, current treatments of tumors in particular fibroids are mainly surgical and expensive. It is then essential to develop and evaluate alternatives to surgical procedures. Selective progesterone receptor modulators are synthetic compounds that have an agonistic or antagonistic impact on target tissues determined by their progesterone receptor binding. Their mixed activity depends on the recruitment of cofactors that regulate transcription along genomic pathways, as well as nongenomic interactions with other signaling pathways. There is no doubt that surgery remains indicated in some cases, but we now need to establish whether the use of selective progesterone receptor modulators can compensate for surgery.

However, the improvement of management must include: raising women's awareness of the clinical manifestations of uterine fibroids and its complications, and insisting on the importance of early management; restructuring or even computerizing the archiving system in order to improve patient follow-up, but also to have an overview of the real incidence of the pathology at national level.

Acknowledgements

The authors are most grateful to all Senegalese women who participated in the present study.

We are extremely grateful to Dr. Daouda CISS and Dr. Alfousseyne GAYE who helped with the collection of samples. Also Pr. SEMBENE the head of Genetic and Population Management Team for all the molecular studies done.

References

- [1] Wathie F. K., 2007. Uterine fibroids at the Roi Baudouin health center in Guédiawaye: about 148 operated cases, Medical thesis. FMPOS: UCAD - Dakar, 188p.
- [2] Bernard P., 2002. Uterine fibromyomas, Corpus Médical-Faculté de Médecine de Grenoble: Université Joseph Fourier.
- [3] Baird D. D., Dunson D. B., Hill M. C., Cousins D., Schectman J. M., 2003. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence, *Am. J. Obstet. Gynecol.*; 188: 100-107.

- [4] Chahal N., Demmouche A., 2013. Epidemiological profile of uterine fibroids in the region of Sidi Bel Abbes, Algeria. *Pan African Medical Journal*; 1-8.
- [5] Eltoukhi H. M., Modi M. N., Weston M., Armstrong A. Y., Stewart E. A., 2014. The Health Disparities of Uterine Fibroids for African American Women: A Public Health Issue, *Am. J. Obstet. Gynecol.*; 210 (3): 194-199.
- [6] Sparic R., M. D., Ljiljana Mirkovic, Ph.D., Antonio Malvasi, M. D., Andrea Tinelli, Ph.D., 2014. Epidemiology of Uterine Myomas A Review, *Int J Fertil Steril*. 2016; 9 (4): 424-435.
- [7] Lamien A., 2009. Uterine leiomyomas: Anatomopathological aspects and impact on the endometrium and ovarian hormones, Diploma of Advanced Study (D.E.A) UCAD - Dakar, 72p.
- [8] Sall, 2017. Management of uterine fibroids at the National Hospital of Pikine concerning 419 operated cases, Thesis Medicine. STATE DIPLOMA: UCAD - Dakar, 100p.
- [9] Catherino W. H., Eltoukhi H. M., Al-Hendy A., 2013. Racial and Ethnic Differences in the Pathogenesis and Clinical Manifestations of Uterine Leiomyoma, *Seminars in Reproductive Medicine*; 31 (5): 370-379.
- [10] Kénémé B., 2020. Uterine Fibroids And Breast Adenofibroids: Epidemiology And Impacts Of Genetic Mutations On Their Developmental Physiology, Unique Doctoral Thesis: UCAD-Dakar, 177p.
- [11] Ndime F, 2020. Penetrance of the p53 gene in uterine fibroids in Senegalese women, Master's thesis in Animal Biology: UCAD-Dakar, 36p.
- [12] Hall T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41: 95-98.
- [13] Thompson J. D., Higgins D. G., Gibson T. J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*; 22 (22): 4673-4680.
- [14] Librado P., Rozas J., 2009. DnaSP version 5: a software for comprehensive analysis of DNA polymorphism data, *Bioinform.*; 25: 1451-1452.
- [15] Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S., 2016. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.*; 30: 2725-2729.
- [16] R Development Core Team, "R: A language and environment for statistical computing", R Foundation for Statistical Computing, Vienna, Austria., 2005.
- [17] Excoffier L., Laval G., Schneider S., 2010. Arlequin version 3.1: An integrated software package for population genetics data analysis, *Evol. Bioinform. Online*; 1: 45-50.
- [18] Mäkinen N., Heinonen H., Moore S., Tomlinson I., Spuy Z., Aaltonen L., 2011. MED12 exon 2 mutations are common in uterine leiomyomas from South African patients. *Oncotarget*; (2): 966-969.
- [19] Kénémé B., Ciss D., Ka S., Mbaye F., Dem., Sembène M., 2018. Uterine Fibroids in Senegal: Polymorphism of MED12 Gene and Correlation With Epidemiological Factors, *American Journal of Cancer Research and Reviews*; 2 (4): 1-16.
- [20] Nakajin S., Hall P. F., 1981. Microsomal cytochrome P450 from neonatal pig testis. Purification and properties of a C21 steroid side-chain cleavage system (17 α -hydroxylase-C17,20 lyase). *Journal of Biological Chemistry*; 256: 3871-3876.
- [21] Nakajin S., Takahashi M., Shinoda M., and Hall, P. F., 1985. Cytochrome b5 promotes the synthesis of delta 16-C19 steroids by homogeneous cytochrome P-450 C21 side-chain cleavage from pig testis. *Biochemical & Biophysical Research Communications*; 132 (2): 708-713.
- [22] Zachmann M., Vollmin J. A., Hamilton W., Prader A., 1972. *Clinical Endocrinology*; 1: 369- 385.
- [23] Goebelsmann U., Zachmann M., Davajan V., Israel R., Mestman J. H., Mishell D. R., 1976. *Gynecology Investigation*; 7: 138-156.
- [24] Sasano H., Okamoto M., Mason J. I., Simpson E. R., Mendelson C. R., Sasano N., Silverberg S. G., 1989. Immunolocalization of aromatase, 17 α -hydroxylase and side-chain-cleavage cytochromes P-450 in the human ovary. *Journal of Reproduction and Fertility*; 85: 163-169.
- [25] Hanukoglu I., 1992. Steroidogenic enzymes-structure, function, and role in regulation of steroidhormone biosynthesis. *Journal of Steroid Biochemistry and Molecular Biology*; 43: 779-804.
- [26] Puche C., Jose M., Cabero A., Meseguer A., 2002. Expression and enzymatic activity of the P450c17 gene in human adipose tissue. *European Journal of Endocrinology*; 146: 223-229.
- [27] Akey J. M., Zhang G., Zhang K., Jin L., Shriver M. D., 2002. Interrogating a high-density SNP map for signatures of natural selection. *Genome Research*; 12: 1805-1814.
- [28] Auchus R. J., 2001. The genetics, pathophysiology, and management of human deficiencies of P450c17. *Endocrinology & Metabolism Clinics of North America*; 30: 101-119.
- [29] Wickenheisser J. K., Quinn P. G., Nelson V. L., Legro R. S., Strauss J. F., McAllister J. M., 2000. Differential activity of the cytochrome P450 17- α -hydroxylase and steroidogenic acute regulatory protein gene promoters in normal and polycystic ovary syndrome theca cells. *Journal of Clinical Endocrinology & Metabolism*; 85: 2304-2311.
- [30] Carey A. H., Dawn Waterworth., Kirty Patel., Davinia White., Julie Little., Patricia Novelli., Stephen Franks., Robert Williamson., 1994. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17, *Human Molecular Genetics*; 3 (10): 1873-1876.
- [31] Sharp L., Cardy A. H., Cotton S. C., Little J., 2004. CYP17 gene polymorphisms: prevalence and associations with hormone levels and associated factors. *Am. J. Epidemiol*; 160 (8): 729-740.
- [32] Li T. C., Tuckerman E. M., Laird S. M., 2002. Endometrial factors in recurrent miscarriage. *Human Reproductive Update*; 8: 43-52.
- [33] Sata F., H. Yamada., A. Yamada., E. H. Kato., S. Kataoka., Y. Saijo., T. Kondo., J. Tamaki., H. Minakami., R. Kishi., 2003. A polymorphism in the CYP17 gene relates to the risk of recurrent pregnancy loss, *Molecular Human Reproduction*; 9 (11): 725-728.

- [34] Amant F., Dorfling C. M., de Brabanter J., Vandewalle J., Vergote I., Lindeque B. G., 2004. A possible role of the cytochrome P450-c17 α gene (*CYP17 α*) polymorphism in the pathobiology of uterine leiomyomas from black South African women: a pilot study. *Acta Obstet. Gynecol. Scand.*; 83: 234–239.
- [35] Vieira L. C. E., Gomes M. T. V., Castro R. A., de Souza N. C. N., da Silva I. D. C. G., Baracat E. C., 2008. Association of CYP17 gene polymorphism with risk of uterine leiomyoma in Brazilian women, *Gynecol. Endocrinol.*; 24 (7): 373–377.
- [36] Alleyne A. T., Austin S., Williams A., 2014. Distribution of *CYP17 α* polymorphism and selected physiochemical factors of uterine leiomyoma in Barbados, *Met. Gene*; 2: 358–365.
- [37] Müssig K., Kaltenbach S., Machicao F., Maser-Gluth C., Hartmann M. F., Wudy S. A., Schnauder G., Haring H. U., Seif F. J., Gallwitz B., 2005. 17 α hydroxylase/17,20 lyase deficiency caused by a novel homozygous mutation (Y27stop) in the cytochrome CYP17 gene. *The Journal of Clinical Endocrinology & Metabolism*; 90 (7): 4362-4365.
- [38] Hyder S. M., Huang J. C., Nawaz Z., Boettger-Tong H., Makela S., Chiappetta C., Stancel G. M., 2000. Regulation of vascular endothelial growth factor expression by estrogens and progestins. *Environmental Health Perspectives*; 108: 785-790.
- [39] Wu X., Serna V. A., Thomas J., Qiang W., Blumenfeld M. L., Kurita T., 2017. Subtype-Specific Tumor-Associated Fibroblasts Contribute to the Pathogenesis of Uterine Leiomyoma. *Cancer Research*; 77 (24): 1-11.
- [40] Yamane T., Inoue Y., Kobayashi-Hattori K., Oishi Y., 2016. Effects of an Amino acid Deficiency on Hyaluronan Synthesis in Human Dermal Fibroblasts, *Food Science and Technology Research*; 22 (2): 279-281.
- [41] Moro J., Tomé D., Schmidely P., Demersay T. C., Azzout-Marniche D., 2020. Histidine: A Systematic Review on Metabolism and Physiological Effects in Human and Different Animal Species, *MDPO*; 12: 1-20.
- [42] Pedroso A. B. J., Zampieri T. T., Donato J., 2015. Reviewing the Effects of L-Leucine Supplementation in the Regulation of Food Intake, Energy Balance, and Glucose Homeostasis, *ISSN*; 7: 3914-3937.
- [43] Lim J. M., Kim G., Rodney L., Levine R. L., 2019. Methionine in Proteins: It's Not Just for Protein Initiation Anymore, *Neurochemical Research*; 44: 247–257.
- [44] Kim J. J., Kurita T., Bulun S. E., 2013. Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. *Endocrinology Reviews*; 34: 130-162.
- [45] Moravek M. B., Yin P., Ono M., Coon J. St., Dyson M. T., Navarro A., Marsh E. E., Chakravarti D., Kim J. J., Wei J. J. 2015. Ovarian steroids, stem cells and uterine leiomyoma: therapeutic implications. *Human Reproduction Updates*; 21: 1-12.
- [46] Andersen J., Dyreyes V. M., Barbieri R. L., Coachman D. M., Miksicek R. J., 1995. Leiomyoma primary cultures have elevated transcriptional response to estrogen compared with autologous myometrial cultures. *Journal of the Society for Gynecologic Investigation*; 2: 542-551.
- [47] Pedetour F., Quade B. J., Weremowicz S., Dal Cin P., Ali S., Morton C. C., 1998. Localization and expression of the human estrogen receptor beta gene in uterine leiomyomata. *Genes Chromosomes Cancer*; 23: 361-366.
- [48] Yin X. J., Wang G., Khan-Dawood F. S., 2007. Requirements of phosphatidylinositol-3 kinase and mammalian target of rapamycin for estrogen-induced proliferation in uterine leiomyoma and myometrium-derived cell lines. *American Journal of Obstetrics and Gynecology*; 196 (176): 171-175.
- [49] Kim J. J., Sefton E. C., 2012. The role of progesterone signaling in the pathogenesis of uterine leiomyoma. *Molecular and Cellular Endocrinology*; 358: 223-231.
- [50] Ishikawa H., Ishi K., Serna V. A., Kakazu R., Bulun S. E., Kurita T., 2010. Progesterone is essential for maintenance and growth of uterine leiomyoma. *Endocrinology*; 151: 2433-2442.
- [51] Hanahan D., Coussens L. M., 2012. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell*; 21: 309-322.