Pathogenicity of CYP17α mutations in the occurrence of uterine fibroids and breast fibroadenomas

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Abstract

Background. Uterine fibroids and breast fibroadenomas are a real public health problem. Despite the efforts made, the etiological factors are still unknown. In addition, these tumors share histopathological similarities, including a higher prevalence in black women. To better understand the factors involved, we set out to assess the genetic characteristics of uterine fibroids and breast fibroadenomas in Senegalese women using CYP17α genes.

Results. For the CYP17α gene, 196 mutations were found among which, 65 are specific to uterine fibroids, 112 mutations specific to breast fibroadenomas and 19 common to both pathologies. Analysis of the pathogenicity of missense mutations reveals that many mutations are considered to be a simple polymorphism and 21 non-synonymous mutations appear to be potentially damaging, including 6 specific to patients with uterine fibroids; 14 found in patients with breast fibroadenomas.

Conclusion. As a genetic marker of risk, the codons 12, 17, 20, 60 and 72 of CYP17α can be a tool to identify high-risk groups of women for prevention and treatment protocols.

Keywords: Fibroids; Uterus; Fibroadenoma; Breast; Genetics; Biomarker; Senegal.

1. Introduction

Uterine fibroids affect 20 to 25% of women in reproductive activity and almost 70% of aged 50 years [1]. According to Okolo [2], fibroids affect millions of women around the world and occur, in 60% of cases, in women aged 45 years. This prevalence varies by race, with women of African ethnicity being at greater risk. Often asymptomatic, uterine fibroids are associated with significant morbidity and constitute a real public health problem. The cost of treatment is excessively expensive and the only treatment deemed effective is surgery [3, 4]. The total costs (including lost productivity and associated morbidities) associated with the surgical and non-surgical treatment of fibroids are estimated to be between US $ 6 billion and $ 35 billion per year [5].

Breast fibroadenomas represent the most frequent benign pathology of the mammary gland [6]. They represent 50% of all biopsies from benign breast tumors, and this rate rises to 75% for biopsies in women under 20 years [6]. Breast fibroadenomas affects particularly women aged 15-35 [7]. According to Nelson et al. [8], 90% of Chinese women with breast fibroadenomas are under 45 years while only 2% are over 50 years.
Uterine fibroids and breast fibroadenomas share similarities including higher prevalence in black women, dependence on steroid hormones, accumulation of extracellular matrix, among others [9-11].

Despite the efforts made, the factors involved in the initiation of both uterine fibroids and breast fibroadenomas remain unknown, but genetic factors appear to be the most likely determinants. There are several clinical and epidemiological observations which suggest that genetic or chromosomal alterations play a significant role in the physiopathology of uterine fibroids and breast fibroadenomas. For example, ethnicity has a major influence on the development and clinical severity of these two pathologies. Since these ethnic disparities cannot be fully explained by socio-economic or environmental factors, research on genetic contributors seems essential.

2. Material and methods

2.1. Samples

Women with uterine fibroids (70 patients) and breast fibroadenomas (43 patients) were recruited from Idrissa Pouye General Hospital and Joliot Curie Institute Dantec Hospital. After obtaining informed consent, each patient was given a biopsy of the tumour tissue and a blood sample (to serve as a control) on an EDTA tube. To guarantee the anonymity of the patients and thus the respect of the professional secrecy, the samples were coded UF (Uterine fibroids) and FA (FibroAdenomas).

2.2. DNA extraction and PCR-sequencing

Total DNA from each sample was extracted using the Qiagen protocol (Qiagen Dneasy Blood and Tissue Kit) according to the manufacturer’s recommendations. PCR conditions were optimized for each pair of primers and applied uniformly for all samples. The amplifications are carried out in a reaction volume of 50 μl. The promoter region (5’UTR) and exon 1 of the CYP17α gene were amplified. The composition of the reaction mixture is given in Table 1. For the electrophoretic migration, 5 μl of PCR product were mixed with 3 μl of bromophenol blue and the mixture was deposited on a 1.5% agarose gel (1.5 g of agarose powder dissolved in 100 ml of TAE buffer) and migrated at 100 volts for 25 minutes.

Table 1 Primers used and gene amplification conditions

| Primers          | Conditions                                      |
|------------------|-------------------------------------------------|
| CYP17α Forward   | 94°C/12 min; 35 cycles à 94°C/30 s, 60°C/30 s, 72°C/1 min; 72°C/7 min |
| CYP17α Reverse   |                                                 |

The sequencing reactions were carried out in a thermal cycler of the MJ Research PTC-225 Peltier type with the ABI PRISM BigDye TM Terminator Cycle kits. Each sample was sequenced using the forward primer. The fluorescent fragments were purified with the BigDye Xterminator purification protocol. The samples were suspended in distilled water and subjected to electrophoresis in ABI 3730xl sequencer (Applied Biosystems).

2.3. Mutations detection

To determine the presence of any mutation as well as its position in relation to the genes, the raw sequencing data for each of the genes was submitted to the Mutation Surveyor software version 5.0.1 which compares the chromatograms submitted with the sequence of reference. For the CYP17α gene, the chromatograms were compared to the reference sequence NT_030059_104589788.

Mutation Surveyor software offers excellent precision and sensitivity as well as low false positive and false negative rates in DNA analysis. The mutation score indicates the level of confidence that a mutation is called a "real mutation". The score is based on the concept of Phred scores, where the quality scores are logarithmically related to the probabilities of error. Here we only consider a mutation if Phred score ≥ 20, value for which the precision is 99%.

The mutations thus detected are then submitted to the Alamut Visual database, which is software to help in the interpretation of genetic mutations identified in the human genome. For each mutation, the HGVS (Human Genome Variation Society) nomenclature, the location, the nature and the effect on the coding sequence are given. If the variation is known and reported in general populations, information regarding some databases of mutations such as dbSNP is also given.
2.4. Prediction of the pathogenicity of mutations

To see if the non-synonymous substitutions of exon 1 of the CYP17α gene are benign or deleterious in cases of uterine fibroids on the one hand and breast fibroadenomas on the other hand, the nucleotide sequences were translated using MEGA7 software [12] into protein sequences and the latter were subjected to SIFT, Mutation Taster and Polyphen-2 software.

SIFT (Sorting Intolerant From Tolerant) software uses sequence homology to predict whether an amino acid substitution will affect the function of the protein and, therefore, potentially alter the phenotype. It considers the position at which the change occurred and the type of amino acid change. Since this is protein sequence, SIFT chooses related proteins and obtains an alignment of these proteins with the query. Based on the amino acids appearing at each position in the alignment, it calculates the probability that an amino acid at one position is tolerated, provided that the most common amino acid after alignment is tolerated. If this normalized value is less than a cutoff value, the substitution should be deleterious [13]. All mutations with a probability score less than 0.05 are considered deleterious; those with a score greater than or equal to 0.05 are considered tolerated.

Mutation Taster software uses a Bayes classifier to possibly predict the pathological potential of an alteration. The Bayes classifier is fed with the result of all the tests and the characteristics of the modifications and calculates the probabilities that this modification is a mutation of the disease or a harmless polymorphism [14]. If an alteration is a "true" SNP it is automatically predicted a "polymorphism". Alterations causing a premature termination codon and ultimately leading to meaningless mRNA are automatically assigned the status "causing disease".

Polyphen-2 software, is also a probabilistic classifier which calculates the functional significance of an allele change by Naive Bayes, a set of supervised learning algorithms to identify deleterious mutations [15]. The entry options for this method are the database acquisition number or protein sequence and variant details. Any mutation with a score ≥ 0.9 (independent number of specific positions) is classified as a "potentially damaging mutation".

In our study, any mutation is considered to be certainly pathogenic if the three software programs predict it to be such.

3. Results

3.1. Nature and position of mutations

Analysis of the chromatograms with the Mutation Surveyor software indicated that the majorities of mutations are localized in tumor tissues. 196 mutations were found (Table 2) among which, 65 are specific to uterine fibroids, 112 mutations specific to breast fibroadenomas and 19 common to both pathologies (c.-43C>A, c.-40C>A, c.-34T>C, c.-15C>T, c.3G>A, c.32C>T, c.62G>A, c.66C>G, c.80A>C, c.81C>A, c.108G>C, c.129C>G, c.134G>A, c.138C>A, c.138C>T, c.171G>T, c.188T>G, c.189C>G, c.195G>T). Of all the mutations found, 167 are new variants and 29 are listed in the dbSNP database.

The c.-34T>C mutation which is located in the 5 'promoter region 34 bp upstream of the translation initiation site has been found in both patients with uterine fibroids and those with breast fibroadenomas. Depending on the effect of polymorphism on coding, 3 variants create an acceptor intron splice site. These are the c.-14G>A, c.-14G>C and c.-12C>G mutations, all found in cases of breast fibroadenomas.

The majority of mutations in exon 1 of the CYP17α gene are non-synonymous mutations (102). Among these non-synonymous mutations, 60 (58.82%) are found in patients with breast fibroadenomas, 33 (32.35%) in patients with uterine fibroids and 9 (8.9%) common to both pathologies. These are the variants p.Thr11Ile, p.Arg21Lys, p.Cys22Trp, p.Tyr27Ser, p.Arg45Lys, p.His46Gln, p.Gln57His, p.Ile63Ser and p.Ile63Met.
| Mutations     | dbSNP            | Effect on coding region                  | Amino acid affected | Pathology |
|--------------|------------------|------------------------------------------|---------------------|-----------|
| c.-93G>T     | New variant      | No effect on the splice site             |                     | UF        |
| c.-86C>T     | New variant      | No effect on the splice site             |                     | UF        |
| c.-81A>T     | New variant      | No effect on the splice site             |                     | FA        |
| c.-77G>C     | New variant      | No effect on the splice site             |                     | UF        |
| c.-76G>T     | New variant      | No effect on the splice site             |                     | FA        |
| c.-75C>G     | New variant      | No effect on the splice site             |                     | UF        |
| c.-73T>A     | New variant      | No effect on the splice site             |                     | UF        |
| c.-60G>A     | New variant      | No effect on the splice site             |                     | FA        |
| c.-58G>A     | rs61754261       | No effect on the splice site             |                     | FA        |
| c.-49G>T     | New variant      | No effect on the splice site             |                     | UF        |
| c.-46C>A     | New variant      | No effect on the splice site             |                     | UF        |
| c.-45T>A     | New variant      | No effect on the splice site             |                     | FA        |
| c.-43C>G     | New variant      | No effect on the splice site             |                     | FA        |
| c.-43C>A     | New variant      | No effect on the splice site             |                     | UF/FA     |
| c.-40C>A     | New variant      | No effect on the splice site             |                     | UF/FA     |
| c.-40C>G     | New variant      | No effect on the splice site             |                     | FA        |
| c.-38C>A     | New variant      | No effect on the splice site             |                     | FA        |
| c.-34T>A     | rs743572         | Strongly activated cryptic site          |                     | UF        |
| c.-34T>C     | rs743572         | Creation of a CCACC box site            |                     | UF/FA     |
| c.-32C>A     | New variant      | No effect on the splice site             |                     | FA        |
| c.-32C>G     | New variant      | No effect on the splice site             |                     | FA        |
| c.-31T>C     | rs1237281550     | No effect on the splice site             |                     | UF        |
| c.-30G>C     | New variant      | No effect on the splice site             |                     | UF        |
| c.-28C>T     | New variant      | No effect on the splice site             |                     | UF        |
| c.-24G>C     | New variant      | No effect on the splice site             |                     | UF        |
| c.-21G>T     | New variant      | No effect on the splice site             |                     | UF        |
| c.-17G>C     | New variant      | No effect on the splice site             |                     | UF        |
| c.-15C>T     | rs140012815      | Weakly activated cryptic site            |                     | UF/FA     |
| c.-14G>A     | rs17115125       | Creation of an acceptor site            |                     | FA        |
| c.-14G>C     | New variant      | Creation of an acceptor site            |                     | FA        |
| c.-12C>G     | New variant      | Creation of an acceptor site            |                     | FA        |
| c.-10C>G     | New variant      | No effect on the splice site             |                     | FA        |
| c.-7A>C      | New variant      | No effect on the splice site             |                     | FA        |
| c.-7A>G      | rs772535358      | No effect on the splice site             |                     | FA        |
| SNP   | Type            | Effect                          | Description                  | Impact |
|-------|-----------------|---------------------------------|-------------------------------|--------|
| c.5C>G | New variant     | Strongly activated cryptic site |                               | FA     |
| c.5C>A | New variant     | Strongly activated cryptic site |                               | UF     |
| c.4C>A | New variant     | Strongly activated cryptic site |                               | FA     |
| c.4C>G | New variant     | Strongly activated cryptic site |                               | FA     |
| c.2C>A | New variant     | Strongly activated cryptic site |                               | FA     |
| c.2C>G | New variant     | Strongly activated cryptic site |                               | FA     |
| c.1A>G | New variant     | Loss of Met initiator codon     |                               | p.Met1Gly | FA |
| c.1A>T | New variant     | Loss of Met initiator codon     |                               | p.Met1Leu | FA |
| c.2T>A | New variant     | Loss of Met initiator codon     |                               | p.Met1Lys | UF |
| c.3G>A | rs61754262      | Loss of Met initiator codon     |                               | p.Met1le | UF/FA |
| c.3G>C | New variant     | Loss of Met initiator codon     |                               | p.Met1Ile | FA |
| c.5G>A | New variant     | Nonsense                        |                               | p.Trp2* | UF |
| c.8A>C | New variant     | Non-synonymous                  |                               | p.Glu3Ala | FA |
| c.8A>T | New variant     | Non-synonymous                  |                               | p.Glu3Val | FA |
| c.12C>T | rs565323692     | Synonymous                      |                               | p.Leu4Leu | FA |
| c.14T>A | New variant     | Non-synonymous                  |                               | p.Val5Glu | FA |
| c.14T>G | rs886171252     | Non-synonymous                  |                               | p.Val5Gly | FA |
| c.15G>T | New variant     | Synonymous                      |                               | p.Val5Val | UF |
| c.24G>A | New variant     | Synonymous                      |                               | p.Leu8Leu | FA |
| c.32C>T | rs72559703      | Non-synonymous                  |                               | p.Leu12Gln | FA |
| c.35T>A | New variant     | Non-synonymous                  |                               | p.Leu12Pro | FA |
| c.37G>A | New variant     | Non-synonymous                  |                               | p.Ala13Thr | FA |
| c.37G>C | New variant     | Non-synonymous                  |                               | p.Ala13Pro | FA |
| c.38C>A | New variant     | Non-synonymous                  |                               | p.Ala13Asp | UF |
| c.41A>G | New variant     | Non-synonymous                  |                               | p.Tyr14Cys | FA |
| c.41A>T | New variant     | Non-synonymous                  |                               | p.Tyr14Phe | FA |
| c.42T>A | New variant     | Nonsense                        |                               | p.Tyr14* | FA |
| c.42T>C | New variant     | Synonymous                      |                               | p.Tyr14Tyr | FA |
| c.51G>A | New variant     | Nonsense                        |                               | p.Trp17* | FA |
| c.52C>T | New variant     | Non-synonymous                  |                               | p.Pro18Ser | FA |
| c.55A>C | New variant     | Non-synonymous                  |                               | p.Lys19Gln | FA |
| c.55A>G | New variant     | Non-synonymous                  |                               | p.Lys19Glu | FA |
| c.56A>G | New variant     | Non-synonymous                  |                               | p.Lys19Arg | FA |
| c.56A>T | New variant     | Non-synonymous                  |                               | p.Lys19Met | FA |
| c.57G>A | New variant     | Synonymous                      |                               | p.Lys19Lys | FA |
| c.57G>C | New variant     | Non-synonymous                  |                               | p.Lys19Asn | FA |
| c.58A>C | New variant     | Synonymous                      |                               | p.Arg20Arg | FA |
| c.58A>G | New variant | Non-synonymous | p.Arg20Gly | FA |
| c.59G>A | New variant | Non-synonymous | p.Arg20Lys | FA |
| c.59G>T | New variant | Non-synonymous | p.Arg20Ile | FA |
| c.61A>G | rs998824476 | Non-synonymous | p.Arg21Gly | FA |
| c.61A>T | New variant | Non-synonymous | p.Arg21Trp | FA |
| c.62G>A | rs61754263 | Non-synonymous | p.Arg21Lys | FA |
| c.64T>A | New variant | Non-synonymous | p.Cys22Arg | FA |
| c.64T>C | rs781329931 | Non-synonymous | p.Cys22Ser | FA |
| c.66C>G | rs762563 | Non-synonymous | p.Cys22Trp | FA |
| c.68C>T | rs368405367 | Non-synonymous | p.Pro23Leu | FA |
| c.70G>T | New variant | Non-synonymous | p.Gly24Cys | UF |
| c.72T>A | New variant | Synonymous | p.Gly24Gly | FA |
| c.72T>G | New variant | Synonymous | p.Gly24Gly | FA |
| c.73G>C | New variant | Non-synonymous | p.Ala25Pro | UF |
| c.77A>G | New variant | Non-synonymous | p.Lys26Arg | FA |
| c.77A>T | New variant | Non-synonymous | p.Lys26Met | FA |
| c.79T>C | New variant | Non-synonymous | p.Tyr27His | UF |
| c.80A>C | rs757251521 | Non-synonymous | p.Tyr27Ser | FA |
| c.80A>C | rs757251521 | Non-synonymous | p.Tyr27Ser | FA |
| c.81C>A | rs104894152 | Nonsense | p.Tyr27* | UF/FA |
| c.86A>C | New variant | Non-synonymous | p.Lys29Thr | UF |
| c.87G>A | New variant | Synonymous | p.Lys29Lys | UF |
| c.88A>G | New variant | Non-synonymous | p.Ser30Gly | UF |
| c.89G>A | rs1341416067 | Non-synonymous | p.Ser30Asn | UF |
| c.90C>A | New variant | Non-synonymous | p.Ser30Arg | UF |
| c.91C>G | New variant | Non-synonymous | p.Leu31Val | UF |
| c.94C>A | New variant | Non-synonymous | p.Leu32Met | UF |
| c.96G>A | New variant | Synonymous | p.Leu32Leu | FA |
| c.96G>T | New variant | Synonymous | p.Leu32Leu | FA |
| c.96G>C | rs1398304295 | Synonymous | p.Leu32Leu | UF |
| c.98C>A | New variant | Non-synonymous | p.Ser33Tyr | UF |
| c.99C>T | rs939625154 | Synonymous | p.Ser33Ser | UF |
| c.100C>A | New variant | Non-synonymous | p.Leu34Met | FA |
| c.100C>T | New variant | Synonymous | p.Leu34Leu | FA |
| c.100C>G | New variant | Non-synonymous | p.Leu34Val | UF |
| c.102C>G | New variant | Synonymous | p.Leu34Leu | UF |
| c.106C>G | New variant | Non-synonymous | p.Leu36Val | UF |
| c.108G>A | rs112892739 | Synonymous | p.Leu36Leu | FA |
| Variant | Type         | Description                               | Gene | Position | Effect | Location |
|---------|--------------|-------------------------------------------|------|----------|--------|----------|
| c.108G>C | New variant  | Synonymous                                | p.Leu36Leu | UF/FA    |
| c.109G>T | New variant  | Non-synonymous                            | p.Val37Leu | UF      |
| c.111G>A | New variant  | Creation of a splicing site              | p.Val37Val | UF      |
| c.112G>A | New variant  | Non-synonymous                            | p.Gly38Ser | UF      |
| c.112G>T | New variant  | Non-synonymous                            | p.Gly38Cys | UF      |
| c.113G>A | New variant  | Non-synonymous                            | p.Gly38Asp | FA      |
| c.113G>T | New variant  | Non-synonymous                            | p.Gly38Val | FA      |
| c.115A>C | New variant  | Non-synonymous                            | p.Ser39Arg | FA      |
| c.115A>G | New variant  | Non-synonymous                            | p.Ser39Gly | FA      |
| c.116G>A | New variant  | Non-synonymous                            | p.Ser39Asn | FA      |
| c.116G>T | New variant  | Non-synonymous                            | p.Ser39Ile | FA      |
| c.120G>C | New variant  | Synonymous                                | p.Leu40Leu | UF      |
| c.121C>G | New variant  | Non-synonymous                            | p.Pro41Ala | UF      |
| c.129C>A | New variant  | Synonymous                                | p.Leu43Leu | FA      |
| c.129C>G | New variant  | Synonymous                                | p.Leu43Leu | UF/FA   |
| c.132C>A | New variant  | Synonymous                                | p.Pro44Pro | FA      |
| c.132C>G | New variant  | Synonymous                                | p.Pro44Pro | FA      |
| c.134G>A | New variant  | Non-synonymous                            | p.Arg45Lys | UF/FA   |
| c.134G>C | New variant  | Non-synonymous                            | p.Arg45Thr | FA      |
| c.137A>G | New variant  | Non-synonymous                            | p.His46Arg | UF      |
| c.138C>C | New variant  | Synonymous                                | p.His46Gln | UF/FA   |
| c.138C>T | rs6162       | Synonymous                                | p.His46His | UF/FA   |
| c.158T>A | New variant  | Non-synonymous                            | p.Phe53Tyr | FA      |
| c.158T>G | New variant  | Non-synonymous                            | p.Phe53Cys | FA      |
| c.141C>A | New variant  | Synonymous                                | p.Gly47Gly | UF      |
| c.142C>T | New variant  | Non-synonymous                            | p.His48Tyr | UF      |
| c.144T>A | New variant  | Non-synonymous                            | p.His48Gln | UF      |
| c.147G>A | New variant  | Non-synonymous                            | p.Met49Ile | UF      |
| c.154A>T | New variant  | Non-synonymous                            | p.Asn52Tyr | UF      |
| c.156C>A | New variant  | Non-synonymous                            | p.Asn52Lys | UF      |
| c.159C>T | New variant  | Synonymous                                | p.Phe53Phe | UF      |
| c.163A>G | New variant  | Non-synonymous                            | p.Lys55Gly | FA      |
| c.163A>T | New variant  | Nonsense                                  | p.Lys55*   | FA      |
| c.164A>T | New variant  | Non-synonymous                            | p.Lys55Met | UF      |
| c.165G>A | New variant  | Synonymous                                | p.Lys55Lys | FA      |
| c.165G>T | New variant  | Non-synonymous                            | p.Lys55Asn | FA      |
| c.167T>A | New variant  | Non-synonymous                            | p.Leu56Gln | UF      |
| c.171G>A | New variant  | Synonymous                                | p.Gln57Gln | FA      |
| c.171G>T | New variant | Non-synonymous | p.Gln57His | UF/FA |
| c.178T>A | New variant | Non-synonymous | p.Tyr60Asn | FA |
| c.178T>C | New variant | Non-synonymous | p.Tyr60His | FA |
| c.179A>G | New variant | Non-synonymous | p.Tyr60Cys | UF |
| c.180T>C | rs61754264 | Synonymous | p.Tyr60Tyr | FA |
| c.180T>G | New variant | Nonsense | p.Tyr60* | UF |
| c.183C>G | New variant | Synonymous | p.Gly61Gly | UF |
| c.185C>T | New variant | Non-synonymous | p.Pro62Leu | FA |
| c.187A>G | New variant | Non-synonymous | p.Ile63Val | FA |
| c.188T>C | New variant | Non-synonymous | p.Ile63Thr | FA |
| c.188T>G | New variant | Non-synonymous | p.Ile63Ser | UF/FA |
| c.189C>G | New variant | Non-synonymous | p.Ile63Met | UF/FA |
| c.190T>C | New variant | Non-synonymous | p.Tyr64His | FA |
| c.190T>G | New variant | Non-synonymous | p.Tyr64Asp | FA |
| c.191A>G | New variant | Non-synonymous | p.Tyr64Cys | FA |
| c.191A>C | New variant | Non-synonymous | p.Tyr64Ser | FA |
| c.193T>A | New variant | Non-synonymous | p.Ser65Thr | FA |
| c.193T>C | New variant | Non-synonymous | p.Ser65Pro | FA |
| c.194C>A | New variant | Synonymous | p.Ser65Ser | UF |
| c.195C>G | New variant | Synonymous | p.Ser65Ser | UF |
| c.195G>A | rs6163 | Synonymous | p.Ser65Ser | FA |
| c.195G>T | rs6163 | Synonymous | p.Ser65Ser | UF/FA |
| c.197T>A | New variant | Non-synonymous | p.Val66Asp | UF |
| c.199C>A | New variant | Non-synonymous | p.Arg67Ser | UF |
| c.200G>A | rs376074317 | Non-synonymous | p.Arg67His | UF |
| c.201T>A | New variant | Synonymous | p.Arg67Arg | FA |
| c.201T>C | New variant | Synonymous | p.Arg67Arg | FA |
| c.207C>A | New variant | Synonymous | p.Gly69Gly | FA |
| c.207C>G | New variant | Synonymous | p.Gly69Gly | FA |
| c.209C>A | New variant | Non-synonymous | p.Thr70Asn | FA |
| c.209C>G | New variant | Non-synonymous | p.Thr70Ser | FA |
| c.211A>T | New variant | Nonsense | p.Lys71* | FA |
| c.213G>A | New variant | Synonymous | p.Lys71Lys | UF |
| c.214A>C | New variant | Non-synonymous | p.Thr72Pro | FA |
| c.214A>G | New variant | Non-synonymous | p.Thr72Ala | FA |
| c.215C>A | New variant | Non-synonymous | p.Thr72Asn | UF |
| c.220G>A | New variant | Non-synonymous | p.Val74Met | FA |
| c.224T>A | New variant | Non-synonymous | p.Ile75Asn | FA |
### 3.2. Effect of non-synonymous mutations

The analysis of the pathogenicity of missense mutations reveals that many mutations are considered to be a single polymorphism and 21 non-synonymous mutations appear to be potentially damaging, 6 of which are specific to patients with uterine fibroids (p.Ser30Arg, p.Leu34Val, p.Gly38Ser, p.Leu56Gln, p.Tyr60Cys and p.Ala82Asp); 14 found in patients with breast fibroadenomas (p.Lys26Met, p.Leu34Met, p.Gly38Asp, p.Gly38Val, p.Ser39Arg, p.Ser39Gly, p.Ser39Leu, p.Tyr60Asn, p.Tyr60His, p.Tyr64His, p.Tyr64Asp, p.Tyr64Cys, p.Tyr64Ser and p.Val74Met) and 1 common mutation (p.Gln57His). Among all these mutations, the p.Gly38Ser mutation found in patients with uterine fibroids appears to be the most pathogenic.

#### Table 3 Prediction of the pathogenicity of CYP17α mutations

| Mutations | SIFT (score) | Mutation Taster (score) | Polyphen2 (score) |
|-----------|--------------|------------------------|------------------|
| c.224T>G  | New variant  | Non-synonymous         | p.Ile75Ser       | FA                |
| c.226G>A  | New variant  | Non-synonymous         | p.Val76Ile       | FA                |
| c.226G>C  | New variant  | Non-synonymous         | p.Val76Leu       | FA                |
| c.229G>A  | New variant  | Non-synonymous         | p.Gly77Ser       | FA                |
| c.231C>A  | New variant  | Synonymous             | p.Gly77Gly       | FA                |
| c.231C>T  | New variant  | Synonymous             | p.Gly77Gly       | FA                |
| c.240G>T  | New variant  | Non-synonymous         | p.Gln80His       | UF                |
| c.245C>A  | New variant  | Non-synonymous         | p.Ala82Asp       | UF                |
| c.267G>C  | New variant  | Non-synonymous         | p.Lys89Asn       | UF                |
| SNP     | Amino Acid Change | Classification     | Effect on Function |
|---------|-------------------|--------------------|--------------------|
| c.59G>A | p.Arg20Lys        | Tolerated (0.4)    | Polymorphic (1)    |
| c.59G>T | p.Arg20Ile        | Tolerated (0.4)    | Polymorphic (1)    |
| c.61A>G | p.Arg21Gly        | Tolerated (0.4)    | Polymorphic (1)    |
| c.61A>T | p.Arg21Trp        | Tolerated (0.4)    | Polymorphic (1)    |
| c.64T>A | p.Cys22Arg        | Tolerated (0.53)   | Polymorphic (1)    |
| c.64T>C | p.Cys22Ser        | Tolerated (0.53)   | Polymorphic (1)    |
| c.66C>T | p.Pro23Lue        | Tolerated (0.4)    | Polymorphic (1)    |
| c.70G>T | p.Gly24Cys        | Tolerated (0.4)    | Polymorphic (1)    |
| c.73G>C | p.Ala25Pro        | Tolerated (0.34)   | Polymorphic (0.84) |
| c.77A>T | p.Lys26Arg        | Tolerated (0.21)   | Polymorphic (0.84) |
| c.79T>C | p.Tyr27His        | Tolerated (0.5)    | Polymorphic (0.9)  |
| c.80A>T | p.Tyr27Phe        | Tolerated (0.39)   | Polymorphic (0.8)  |
| c.86A>C | p.Lys29Thr        | Tolerated (0.18)   | Polymorphic (0.9)  |
| c.88G>T | p.Ser30Gly        | Tolerated (0.37)   | Polymorphic (0.9)  |
| c.90C>A | p.Leu34Met        | Tolerated (0.23)   | Polymorphic (0.9)  |
| c.100C>A| p.Leu34Met        | Tolerated (0.1)    | Polymorphic (1)    |
| c.106C>G| p.Leu36Val        | Tolerated (0.06)   | Polymorphic (0.7)  |
| c.113G>A| p.Gly38Ser        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.115A>G| p.Gly38Asp        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.115A>C| p.Gly38Val        | Tolerated (0.06)   | Polymorphic (0.7)  |
| c.116A>G| p.Gly39Arg        | Tolerated (0.06)   | Polymorphic (0.7)  |
| c.116G>A| p.Gly39Lys        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.117G>A| p.Gly39Asn        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.119G>A| p.Gly43Arg        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.119G>A| p.Leu43Met        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.121G>C| p.Leu43Val        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.124G>A| p.Arg45Lys        | Tolerated (0.07)   | Polymorphic (0.9)  |
| c.124G>C| p.Arg45Thr        | Tolerated (0.38)   | Polymorphic (1)    |
| c.137A>G| p.His46Arg        | Tolerated (0.57)   | Polymorphic (1)    |
| Variant               | Effect          | Usage            | Impact           |
|-----------------------|-----------------|------------------|------------------|
| c.138C>A p.His46Gln   | Tolerated (0.38)| Polymorphic (1)  | Possible Damage (0.8) |
| c.142C>T p.His48Tyr   | Tolerated (0.4) | Polymorphic (1)  | Possible Damage (0.7) |
| c.144T>A p.His46Gln   | Tolerated (0.3) | Polymorphic (1)  | Benign (0.0028)  |
| c.147G>A p.Met49Ile   | Tolerated (0.48)| Polymorphic (1)  | Benign (0.37)    |
| c.154A>T p.Asn52Tyr   | Tolerated (1)   | Polymorphic (1)  | Benign (0.037)   |
| c.156C>A p.Asn52Lys   | Tolerated (0.72)| Polymorphic (1)  | Benign (0.037)   |
| c.158T>A p.Phe53Tyr   | Tolerated (1)   | Polymorphic (0.9)| Benign (0.051)   |
| c.158T>G p.Phe53Cys   | Tolerated (0.2) | Polymorphic (0.9)| Benign (0.051)   |
| c.163A>G p.Lys55Glu   | Tolerated (0.2) | Polymorphic (0.5)| Benign (0.012)   |
| c.164A>T p.Lys55Met   | Tolerated (0.08)| Polymorphic (0.9)| Benign (0.051)   |
| c.165G>T p.Lys55Asn   | Tolerated (0.38)| Polymorphic (1)  | Possible Damage (0.8) |
| c.167T>A p.Leu56Gln   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.171G>T p.Gln57His   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.178T>G p.Tyr60Asp   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.179A>G p.Tyr60Cys   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.185C>T p.Pro62Leu   | Tolerated (0.08)| Polymorphic (0.9)| Benign (0.051)   |
| c.187A>G p.Ile63Val   | Tolerated (0.38)| Polymorphic (1)  | Possible Damage (0.8) |
| c.188T>C p.Ile63Thr   | Tolerated (0.38)| Polymorphic (1)  | Possible Damage (0.8) |
| c.188T>G p.Ile63Ser   | Tolerated (0.1) | Polymorphic (0.8)| Possible Damage (0.8) |
| c.189C>G p.Ile63Met   | Tolerated (0.4) | Polymorphic (0.79)| Possible Damage (0.8) |
| c.190T>C p.Tyr64His   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.190T>G p.Tyr64Asp   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.191A>G p.Tyr64Cys   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.191A>C p.Tyr64Ser   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.193T>A p.Ser65Thr   | Tolerated (0.08)| Polymorphic (0.79)| Possible Damage (0.8) |
| c.193T>C p.Ser65Pro   | Tolerated (0.08)| Polymorphic (0.79)| Possible Damage (0.8) |
| c.197T>A p.Val66Asp   | Tolerated (0.1) | Polymorphic (1)  | Possible Damage (0.5) |
| c.200G>A p.Arg67Ser   | Tolerated (0.14)| Polymorphic (0.79)| Possible Damage (0.8) |
| c.200G>A p.Arg67His   | Tolerated (0.15)| Polymorphic (1)  | Possible Damage (0.85) |
| c.209C>A p.Tyr70Asn   | Tolerated (0.15)| Polymorphic (1)  | Possible Damage (0.85) |
| c.209C>G p.Tyr70Ser   | Tolerated (1)   | Polymorphic (1)  | Benign (0.037)   |
| c.214A>C p.Thr72Pro   | Tolerated (1)   | Polymorphic (1)  | Benign (0.037)   |
| c.214A>G p.Thr72Ala   | Tolerated (1)   | Polymorphic (1)  | Benign (0.037)   |
| c.215C>A p.Thr72Asn   | Tolerated (0.45)| Polymorphic (1)  | Possible Damage (0.46) |
| c.220G>A p.Val74Met   | Deleterious (0.01)| Disease causing (1)| Damaging (1)   |
| c.224T>A p.Ile75Asn   | Tolerated (0.08)| Polymorphic (0.9)| Possible Damage (0.5) |
| c.224T>G p.Ile75Ser   | Tolerated (0.08)| Polymorphic (0.9)| Possible Damage (0.5) |
Among the numerous chemical transformations catalyzed by cytochrome P450 enzymes, hydroxylations and cleavages of steroid hormones are of particular interest because of their mechanical complexity and their essential roles in physiology. The human cytochrome P450-17A1 enzyme acts at a key point in human steroidogenesis, controlling levels of mineralocorticoids influencing blood pressure, glucocorticoids involved in immune and stress responses, and androgens involved in development and homeostasis of reproductive tissues [16]. The CYP17A1 enzyme catalyzes both 17α-hydroxylase and 17,20-lyase activities and also has a modest degree of 16α-hydroxylase activity [17]. The steroid 17α-hydroxylase converts pregnenolone to 17-hydroxyprogrenolone and converts progesterone to 17-hydroxyprogesterone, a precursor or mild synthesis of testosterone and estrogen. Although the activities of the 17α-hydroxylase and 17,20-lyase steroids can be easily distinguished, examination of circulating venous steroid products has shown that both activities reside in a single protein, CYP17A1 encoded by the CYP17a gene [18, 19]. Thus, the CYP17A1 enzyme is a key branch point in the synthesis of human steroid hormones.

In females, CYP17α is expressed in adrenal glands, adipose tissue, ovarian thecal cells, and corpus luteum [20]. The CYP17A1 enzyme converts 21-carbon steroids to 19-carbon androgens in two chemical changes [21]. First, pregnenolone or 21-carbon progesterone is hydroxylated. The 17α-hydroxyprogrenolone product can undergo a second 17,20-lyase reaction in the same active site to give 19-carbon dehydroepiandrosterone (DHEA), androgen precursor for all sex steroids. Thus, mutations or clinical inhibition affecting both reactions of CYP17A1 block the production of androgens and glucocorticoids.

In this study, 196 mutations of the CYP17α gene were found among which, 65 are specific to uterine fibroids, 112 mutations specific to breast fibroadenomas and 19 common to both pathologies. Mutations at the level of the promoter region of CYP17α are very frequent both in fibroids and in fibroadenomas, attesting to an altered mechanism in transcription regulation. These mutations could induce synthetic overexpression of the CYP17A1 enzyme in female patients. Indeed, the work of Wickenheisser et al. [22] showed that CYP17A1 expression in theca cells isolated from women with polycystic ovary syndrome is consistently high, compared to normal cells. To study the mechanism of the increased accumulation of CYP17A1 mRNA in ovarian tumor cells, Wickenheisser et al. [22] examined the activities of the CYP17α promoter and the steroidogenic acute regulatory protein (StAR). The activity of the basal CYP17α promoter was 4-fold higher in ovarian tumor cells than in theca cells isolated from normal ovaries. The authors concluded from these data that the transcription of the basal and cAMP-dependent CYP17α gene is increased in tumor cells.

In addition, the c.-34T>C mutation creates a new CCACC box site and therefore an additional promoter. Carey et al. [23] first identified this SNP and hypothesized that the C allele could upregulate gene expression by first increasing serum hormones including androstenedione and estradiol (E2). Several studies have hypothesized that the C allele of CYP17α may be a marker of increased steroidogenesis [24, 25]. Sun et al. [26] concluded that the c.-34T>C mutation could increase the risk of breast cancer in postmenopausal women.

As with the c.195G>A and c.195G>T mutations, the c.-15C>T promoter region variant has been identified in congenital adrenal hyperplasia. These are a group of autosomal recessive disorders comprising enzyme deficiencies in the adrenal steroidogenesis pathway that lead to impaired cortisol biosynthesis. Depending on the type and severity of the steroid block, patients may experience various alterations in the production of glucocorticoids, mineralocorticoids, and sex steroids requiring hormone replacement therapy.

The c.3G>A mutation alters the 1st amino acid of exon 1 of the CYP17α gene, namely methionine and therefore results in the loss of the codon that initiates translation. This loss could result in inhibition of the synthesis of the enzyme CYP17A1 and therefore blockade of 17α-hydroxylase and 17,20-lyase activities in patients with this polymorphism. This is further confirmed by the discovery of the c.5G>A (p.Trp2*) and c.51G>A (p.Trp17*) variants that we found.
respectively in patients with uterine fibroids and those with breast fibroadenomas. These variants correspond to a premature stop codon for Tryptophan in codons 2 (p.W2X) and 17 (p.W17X) and induce the genetic disorder of CYP17A1 which causes a deficiency in 17α-hydroxylase/17,20-lyase. The mutation at position 17 could induce the loss of the C17 cleavage of the CYP17A1 enzyme and thus an overexpression of sex steroids by inhibition of regulatory metabolism. The c.81C>A which corresponds to a replacement of Tyrosine 27 by a stop codon (p.Tyr27*) is also a pathogenic variant found in uterine fibroids as well as in breast fibroadenomas. This polymorphism which creates a truncated enzyme is also found in other pathologies. Indeed, the study of Müssig et al. [27] reported 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 17α-hydroxycorticoids, androgens and estrogen. The postulate is that in this patient, both copies of the 17α-hydroxylase gene are defective in the form of a homozygous stop codon in exon 1, severely truncating the protein at amino acid 27. The heme binding site, the substrate binding pocket, and the redox partner site, all of which have been reported to be essential for C17 catalytic activity, are lacking. Therefore, CYP17A1 will have no activity, neither as a 17α-hydroxylase nor as a 17,20-lyase.

In addition to a high rate of polymorphism of the CYP17α gene, 21 non-synonymous mutations appear to be potentially damaging. These mutations are predicted to be a molecular entity serving as an electron transport chain attesting to their involvement in the enzymatic activities of CYP17A1. This is because 17,20-lyase activity, which involves the oxidative cleavage of a CC bond, is regulated in a tissue-specific manner and programmed by factors such as the abundance of the electron donor flavoprotein P450-oxidoreductase [28, 29], the coexistence of 3b-hydroxysteroid dehydrogenase and P450c21 [30].

In addition to their electron transport activity, deleterious CYP17α mutations induce a modification of the biological function of the enzyme and attest to their involvement in the pathophysiology of uterine fibroids and breast fibroadenomas. The p.Lys26Met mutation is predicted to have a function of DNA-directed RNA polymerase which has the role of catalyzing the transcription of DNA into RNA using the four ribonucleoside triphosphates as substrates. Variants p.S30R, p.L34M, p.G38D, p.S39G, p.S39I, p.L56Q, p.Y60H, p.Y60C, p.Y64H and p.Y64S maintain cytochrome P450 function with variations histochemicals. These cytochromes form a group of heme-thiolate monooxygenases. The p.Lys34Val mutation leads to a ubiquitin E2 enzyme. The latter accepts ubiquitin from the El complex and catalyzes its covalent attachment to other proteins. In vitro, it catalyzes polyubiquitination linked to Lys48 and mediates the selective degradation of abnormal and short-lived proteins [31]. The p.Gly38Ser mutation specific to patients with uterine fibroids appears to be the most pathogenic and is predicted to be part of the ferritins in addition to the p.Y64D and p.V74M variants. Ferritins are intracellular proteins capable of storing thousands of iron (III) ions in the form of a solid mineral [32]. The subunits are characterized by a four-helix bundle structure. Iron (II) enters the ion channels of the protein shell and reaches the active site, known as the ferroxidase site, which is a dinuclear metal ion binding site. The p.Gln57His mutation, which is common to both pathologies, gives the protein the function of 1,25-dihydroxyvitamin-D3-24-hydroxylase, which is a cytochrome P450 monoxygenase with a key role in the catabolism of vitamin D and calcium homeostasis. It has been established over the past decade that CYP11A1 (also known as cytochrome P450sc) can metabolize vitamin D3 to produce many new mono and poly-hydroxyvitamin D metabolites, the main one being 20S-hydroxyvitamin-D3-(20(OH)D3) and 20S,23-dihydroxyvitamin-D3-(20,23(OH)2D3) [33].

5. Conclusion

The results on the molecular bases obtained show a significant alterations of the CYP17α genes in uterine fibroids and breast fibroadenomas. A polymorphism of the CYP17α gene is noted in both fibroids and fibroadenomas, but more marked for breast pathology. Arginine codon 20 being under positive selection in cases of breast fibroadenoma indicate its implication in the pathophysiology of tumour cells.

At the end of this study we can postulate that as a genetic marker of risk, codons 12, 17, 20, 60 and 72 of CYP17α gene can be another tool to identify groups of women at high risk for prevention and treatment protocols.

Compliance with ethical standards

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Disclosure of conflict of interest
The authors declare no competing interests.

Statement of ethical approval
This study was carried out in accordance with the recommendations of World Medical Association’s Declaration of Helsinki. The protocol was approved by the Institutional Ethics Committee on Human Research of Cheikh Anta Diop University (Reference: Protocol 0267/2017/CER/UCAD).

Statement of informed consent
All subjects gave written informed consent according to a standardized form.

Author contributions
BK performed molecular analysis, organized the database, performed data analysis, and wrote the first draft of the manuscript. MS contributed to conception and design of the study, revised the manuscript, and read and approved the submitted version.

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