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Pathogenicity of *CYP17α* mutations in the occurence 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.

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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 5'-TCCTGAGCCCAGATACCAT-3'	94°C/12 min ; 35 cycles à 94°C/30 s, 60°C/30 s, 72°C/1 min ; 72°C/7 min
	Reverse 5'-CCGCCCAGAGAAGTCCT-3'	

The sequencing reactions were carried out in a thermal cycler of the MJ Research PTC-225 Peltier type with the ABIPRISM 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 (Appled 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 majority 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.

Table 2 Position and nature of mutations in the *CYP17α* gene

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.-45T>G	New variant	No effect on the splice site		FA
c.-43C>A	New variant	No effect on the splice site		UF/FA
c.-43C>T	New variant	No effect on the splice site		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		UF
c.-34T>A	rs743572	Strongly activated cryptic site		FA
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

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.Met1Ile	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	UF
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.Thr11Ile	UF/FA
c.35T>A	New variant	Non-synonymous	p.Leu12Gln	FA
c.35T>C	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	rs104894141	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	UF/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	UF/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	UF/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

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>A	New variant	Non-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.195G>C	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

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

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.Ser39Ile, 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.8A>C p.Glu3Ala	Tolerated (0.4)	Polymorphic (0.9)	Benign (0.025)
c.8A>T p.Glu3Val	Tolerated (0.4)	Polymorphic (0.9)	Benign (0.025)
c.14T>A p.Val5Glu	Tolerated (0.07)	Polymorphic (0.9)	Possible Damage (0.5)
c.14T>G p.Val5Gly	Tolerated (0.10)	Polymorphic (0.9)	Possible Damage (0.5)
c.28C>A p.Leu10Ile	Tolerated (0.4)	Polymorphic (0.9)	Possible Damage (0.5)
c.32C>T p.Thr11Ile	Tolerated (0.6)	Polymorphic (1)	Benign (0.025)
c.35T>A p.Leu12Gln	Tolerated (0.6)	Polymorphic (1)	Benign (0.025)
c.35T>C p.Leu12Pro	Tolerated (0.6)	Polymorphic (1)	Benign (0.025)
c.37G>A p.Ala13Thr	Tolerated (0.6)	Polymorphic (1)	Benign (0.025)
c.37G>C p.Ala13Pro	Tolerated (0.4)	Polymorphic (0.9)	Possible Damage (0.5)
c.38C>A p.Ala13Asp	Tolerated (0.4)	Polymorphic (1)	Possible Damage (0.6)
c.41A>G p.Tyr14Cys	Tolerated (0.4)	Polymorphic (0.9)	Possible Damage (0.5)
c.41A>T p.Tyr14Phe	Tolerated (0,4)	Polymorphic (0.9)	Possible Damage (0.5)
c.52C>T p.Pro18Ser	Tolerated (0.34)	Polymorphic (0.9)	Benign (0.37)
c.55A>C p.lys19Gln	Tolerated (0.4)	Polymorphic (0.9)	Possible Damage (0.5)
c.55A>G p.lys19Glu	Tolerated (0.4)	Polymorphic (0.9)	Possible Damage (0.5)
c.56A>G p.Lys19Arg	Tolerated (0.4)	Polymorphic (0.9)	Benign (0.37)
c.56A>T p.Lys19Met	Tolerated (0.4)	Polymorphic (1)	Possible Damage (0.5)
c.57G>C p.Lys19Asn	Tolerated (0.4)	Polymorphic (1)	Possible Damage (0.5)
c.58A>G p.Agr20Gly	Tolerated (0.4)	Polymorphic (1)	Benign (0.37)

c.59G>A p.Arg20Lys	Tolerated (0.4)	Polymorphic (1)	Benign (0.37)
c.59G>T p.arg20Ile	Tolerated (0.4)	Polymorphic (1)	Benign (0.37)
c.61A>G p.Arg21Gly	Tolerated (0.4)	Polymorphic (1)	Benign (0.37)
c.61A>T p.Arg21Trp	Tolerated (0.4)	Polymorphic (1)	Possible Damage (0.59)
c.62G>A p.Arg21Lys	Tolerated (0.53)	Polymorphic (1)	Benign (0.001)
c.64T>A p.Cys22Arg	Tolerated (0.53)	Polymorphic (1)	Benign (0.001)
c.64T>C p.Cys22Ser	Tolerated (0.53)	Polymorphic (1)	Benign (0.001)
c.66C>G p.Cys22Trp	Tolerated (0.4)	Polymorphic (1)	Possible Damage (0.59)
c.68C>T p.Pro23Lue	Tolerated (0.4)	Polymorphic (1)	Possible Damage (0.59)
c.70G>T p.Gly24Cys	Tolerated (0.4)	Polymorphic (1)	Possible Damage (0.5)
c.73G>C p.Ala25Pro	Tolerated (0.34)	Polymorphic (0.84)	Benign (0.221)
c.77A>G p.Lys26Arg	Tolerated (0.21)	Polymorphic (0.84)	Benign (0.221)
c.77A>T p.Lys26Met	Deleterious (0.03)	Disease causing (1)	Damaging (1)
c.79T>C p.Tyr27His	Tolerated (0.5)	Polymorphic (0.9)	Possible Damage (0.5)
c.80A>C p.Tyr27Ser	Tolerated (0.39)	Polymorphic (0.8)	Benign (0.221)
c.80A>T p.Tyr27Phe	Tolerated (0.39)	Polymorphic (0.8)	Benign (0.221)
c.86A>C p.Lys29Thr	Tolerated (0.18)	Polymorphic (0.9)	Benign (0.051)
c.88A>G p.Ser30Gly	Tolerated (0.37)	Polymorphic (0.9)	Benign (0.051)
c.89G>A p.Ser30Asn	Tolerated (0.08)	Polymorphic (0.9)	Benign (0.051)
c.90C>A p.Ser30Arg	Deleterious (0.03)	Disease causing (1)	Damaging (1)
c.91C>G p.Leu31Val	Tolerated (0.17)	Polymorphic (0.9)	Benign (0.051)
c.94C>A p.Leu32Met	Tolerated (0.23)	Polymorphic (0.9)	Benign (0.051)
c.98C>A p.Ser33Tyr	Tolerated (0.1)	Polymorphic (1)	Benign (0.005)
c.100C>A p.Leu34Met	Deleterious (0)	Disease causing (1)	Damaging (0.9)
c.100C>G p.Leu34Val	Deleterious (0)	Disease causing (1)	Damaging (0.9)
c.106C>G p.Leu36Val	Tolerated (0.06)	Polymorphic (0.7)	Benign (0.005)
c.109G>T p.Val37Leu	Tolerated (0.07)	Polymorphic (0.9)	Benign (0.10)
c.112G>A p.Gly38Ser	Deleterious (0)	Disease causing (1)	Damaging (1)
c.113G>A p.Gly38Asp	Deleterious (0)	Disease causing (1)	Damaging (0.9)
c.113G>T p.Gly38Val	Deleterious (0)	Disease causing (1)	Damaging (0.9)
c.115A>C p.Ser39Arg	Deleterious (0)	Disease causing (1)	Damaging (0.9)
c.115A>G p.Ser39Gly	Deleterious (0)	Disease causing (1)	Damaging (0.9)
c.116G>A p.Ser39Asn	Tolerated (0.07)	Polymorphic (0.9)	Benign (0.051)
c.116G>T p.Ser39Ile	Deleterious (0)	Disease causing (1)	Damaging (0.9)
c.121C>G p.Pro41Ala	Tolerated (0.2)	Polymorphic (0.9)	Benign (0.051)
c.134G>A p.Arg45Lys	Tolerated (0.9)	Polymorphic (1)	Benign (0.012)
c.134G>C p.Arg45Thr	Tolerated (0.38)	Polymorphic (1)	Possible Damage (0.8)
c.137A>G p.His46Arg	Tolerated (0.57)	Polymorphic (1)	Benign (0.007)

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.His48Gln	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>A p.Tyr60Asn	Deleterious (0.01)	Disease causing (1)	Damaging (1)
c.178T>C p.Tyr60His	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.199C>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)

c.226G>A p.Val76Ile	Tolerated (1)	Polymorphic (1)	Benign (0.037)
c.226G>C p.Val76Leu	Tolerated (0.14)	Polymorphic (0.79)	Possible Damage (0.8)
c.229G>A p.Gly77Ser	Tolerated (0.08)	Polymorphic (0.9)	Possible Damage (0.7)
c.240G>T p.Gln80His	Tolerated (0.17)	Polymorphic (0.79)	Possible Damage (0.8)
c.245C>A p.Ala82Asp	Deleterious (0.01)	Disease causing (1)	Damaging (1)
c.267G>C p.Lys89Asn	Tolerated (0.34)	Polymorphic (0.79)	Possible Damage (0.8)

4. Discussion

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-hydroxypregnenolone 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 *CYP17α* 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-hydroxypregnenolone 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 thecal 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 monooxygenase 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 P450scc) 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.

References

[1] Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. American Journal of Obstetrics & Gynecology. 2003; 188:100-107.

[2] Okolo S. Incidence, aetiology and epidemiology of uterine fibroids. Best Practice & Research: Clinical Obstetrics & Gynaecology. 2008; 22(4):571-588.

[3] Farquhar CM, Steiner. CA Hysterectomy rates in the United States 1990–1997. Obstetrics & Gynecology. 2002; 99:229-234.

[4] Wu JM, Wechter ME, Geller EJ, Nguyen TV, Visco AG. Hysterectomy rates in the United States, 2003. Obstetrics & Gynecology. 2007; 110:1091-1095.

[5] Cardozo ER, Clark AD, Banks NK, Henne MB, Stegmann BJ, Segars JH. The estimated annual cost of uterine leiomyomata in the United States. American Journal of Obstetrics & Gynecology. 2012; 206:211-219.

[6] Schuerch C, Rosen PP, Hirota T, Itabashi M. A pathologic study of benign breast disease in Tokyo and New York. Cancer. 1982; 50:1899-1902.

[7] Dent DM, Cant PJ. Fibroadenoma. World Journal of Surgery. 1989; 13:706-710.

[8] Nelson ZC, Ray RM, Gao DL, Thomas DB. Risk Factors for Fibroadenoma in a Cohort of Female Textile Workers in Shanghai. American Journal of Epidemiology. 2002; 156(7):599-605.

[9] Tamaya T, Fujimoto J, Okada H. Comparison of cellular levels of steroid receptors in uterine leiomyoma and myometrium. Acta Obstetricia & Gynecologica Scandinavica. 1985; 64:307- 309.

[10] Kjerulff KH, Langenberg P, Seidman JD, Stolley PD, Guzinski GM. Uterine leiomyomas: racial differences in severity, symptoms and age at diagnosis. Journal of Reproductive Medicine. 1996; (7):483-490.

[11] Goffin L, Seguin-Estevez Q, Alvarez M. Transcriptional regulation of matrix metalloproteinase-1 and collagen 1A2 explains the anti-fibrotic effect exerted by proteasome inhibition in human dermal fibroblasts. Arthritis Research & Therapy. 2010; 12:R73.

[12] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology & Evolution. 2013; 30:2725-2729.

[13] Ng PC, Henikoff S. SIFT: prediction amino acid changes that affect protein function. Nucleic Acids Research. 2003; 31(13):3812-3814.

[14] Schwarz JM, Cooper DN, Schuelke M, Seelow D. Mutation Taster2: Mutation prediction for the deep-sequencing age. Nature Methods. 2014; 11(4):361-362.

[15] Adzhubei I, Jordan DM, Sunyaev SR. Prediction functional effect of human missense mutation using Polypen-2. Nature Methods. 2010; 7:248-249.

[16] Nakajin S, Hall PF. Microsomal cytochrome P450 from neonatal pig testis. Purification and properties of a C21 steroid side-chain cleavage system (17α-hydroxylase-C17,20 lyase). Journal of Biological Chemistry. 1981; 256:3871-3876.

[17] Nakajin S, Takahashi M, Shinoda M, Hall PF. Cytochrome b5 promotes the synthesis of delta 16-C19 steroids by homogeneous cytochrome P-450 C21 side-chain cleavage from pig testis. Biochemical and Biophysical Research Communications. 1985; 132(2):708-713.

[18] Zachmann M, Völlmin JA, Mürset G, Curtius HC, Prader A. Unusual type of congenital adrenal hyperplasia probably due to deficiency of 3β-hydroxysteroid dehydrogenase. Case report of a surviving girl and steroid studies. Journal of Clinical Endocrinology and Metabolism. 1970. 30; 719-726.

[19] Goebelsmann U, Zachmann M, Davajan V, Israel R, Mestman JH, Mishell DR. Male pseudohermaphroditism consistent with 17,20-desmolase deficiency. Gynecologic and Obstetric Investigation. 1976; 7:138-156.

[20] Puche C, Jose M, Cabero A, Meseguer A. Expression and enzymatic activity of the P450c17 gene in human adipose tissue. European Journal of Endocrinology. 2002; 146:223-229.

[21] Arlt W, Mertens JW, Song M, Wang JT, Auchus RJ, Miller WL. Molecular evolution of adrenarche: structural and functional analysis of p450c17 from four primate species. Endocrinology. 2002; 143(12):4665-4672.

[22] Wickenheisser JK, Quinn PG, Nelson VL, Legro RS, Strauss JF, McAllister JM. Differential activity of the cytochrome P450 17-alpha-hydroxylase and steroidogenic acute regulatory protein gene promoters in normal and polycystic ovary syndrome theca cells. Journal of Clinical Endocrinology and Metabolism. 2000; 85:2304-2311.

[23] Carey AH, Waterworth D, Patel K., White D, Little J, Novelli P, Franks S, Williamson R. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. Human Molecular Genetics. 1994; 3:1873-1876.

[24] Li TC, Tuckerman EM Laird, SM. Endometrial factors in recurrent miscarriage. Human Reproduction Update. 2002; 8:43-52.

[25] Sata F, Yamada H, Yamada A, Kato EH, Kataoka S, Saijo Y, Kondo T, Tamaki J, Minakami H, Kishi R. A polymorphism in the CYP17 gene relates to the risk of recurrent pregnancy loss. Molecular Human Reproduction. 2003; 9:725-728.

[26] Sun J, Zhang H, Gao M, Tang Z, Guo D, Zhang X, Li R, Liu Y, Sun W, Sun X. Association between CYP17 T-34C rs743572 and breast cancer risk. Oncotarget. 2017; 9(3):4200- 4213.

[27] Müssig K, Kaltenbach S, Machicao F, Maser-Gluth C, Hartmann MF, Wudy SA, Schnauder G, Haring HU, Seif FJ, Gallwitz B. 17α hydroxylase/17,20 lyase defiency caused by a novel homozygous mutation (Y27stop) in the cytochrome CYP17 gene. Journal of Clinical Endocrinology and Metabolism. 2005; 90(7):4362-4365.

[28] Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrinology Reviews. 2011; 32:81-151.

[29] Guengerich FP. Cytochrome P450: Structure, Mechanism, and Biochemistry (Ortiz de Montellano, P R, ed) 4th Ed., 2015; pp 523-785, Springer, New York.

[30] Yoshimoto FK, Auchus RJ. The diverse chemistry of cytochrome P450 17A1 (P450c17, CYP17A1). Journal of Steroid Biochemistry and Molecular Biology. 2015; 151:52-65.

[31] Gonen H, Bercovich B, Orian A, Carrano A, Yamanakai CT, Pagano M, Iwaii K, Ciechanover A. Identification of the Ubiquitin Carrier Proteins, E2s, Involved in Signal- induced Conjugation and Subsequent Degradation of IkBa. The Journal of Biological Chemistry. 1999; 274(21):14823-14830.

[32] Liu X, Theil EC. Ferritins: Dynamic Management of Biological Iron and Oxygen Chemistry. Accounts of Chemical Research. 2005; 38:167-175.

Slominski AT, Kim TK, Li W, Yi AK, Postlethwaite A, Tuckey RC. The role of CYP11A1 in the production of vitamin D metabolites and their role in the regulation of epidermal functions. Journal of Steroid Biochemistry and Molecular Biology. 2014; 144:28-39.