SRYAHWA PUBLICATIONS

Journal of Genetics and Genetic Engineering
ISSN: 2637-5370 | Volume 6, Issue 1, 2024
https://doi.org/10.22259/2637-5370.0601001

RESEARCH ARTICLE

Mutational Penetrance of the CYP17A1 Gene in the Evolution of Uterine Fibroids Coexisting with Pregnancy in Senegalese Women

Bineta Kénémé[1,2], Codou Diop[1*], Ndèye Ka[1], Pape Mbacké Sembene[1,2]

[1]Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal.
[2]Laboratoire de Génomique, Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal.

Received: 18 May 2024 Accepted: 28 May 2024 Published: 10 June 2024

Corresponding Author: Dr. Codou Diop, Génétique des Populations, Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal.

Abstract

Uterine fibroids, also known as myomas or leiomyomas, are the most common solid tumors of the gynecological tract. Symptoms associated with fibroids include excessive uterine bleeding, pelvic pain or pressure, infertility and pregnancy complications. It is not uncommon for fibroids to be diagnosed in pregnant women. They occur in between 3% and 12% of pregnant women, with some data indicating a prevalence of up to 20%. Our aim is to evaluate the evolution of uterine fibroids and to understand the genetic link between fibroids and pregnancy in Senegalese women. We used PCR-sequencing to analyze the impact of non-synonymous variants on the protein and variability of the CYP17A1 gene in 56 patients, including 20 pregnancy women with fibroids and 36 patients with fibroids only. First of all mutations were detected using Mutation Surveyor, then the pathogenicity of non-synonymous variants using in silicotools and the functional impact of non-synonymous variants were also analysed. The secondary and three-dimensional structure of the protein, gene/gene and protein/protein interactions were determined, and finally the sequences obtained were verified, cleaned and aligned. Molecular analyses of the gene showed that the c.-34 T>C polymorphism may be involved in the incidence of the disease and its progression during pregnancy. Five of the non-synonymous variants (p.Val5Gly, p.Ser30Asn, p.Gln57His, p.Val66Asp and p.Arg67His) are pathogenic. Six variants (p.Val5Gly, p.Leu8Val, p. Tyr14Asn, p.Phe16Ile, p.Lys26Thr) and one specific to fibroid tissue and pregnancy (p.Ser30Asn) create genetic disorders in the secondary structure. Co-expression with other genes and functional associations with other proteins are observed. The c.-34T>C polymorphism is involved in fibroid cell growth during pregnancy through the effect of hormone overexpression, while non-synonymous variants lead to dysfunction in protein synthesis and induce a change in the enzyme's biological function.

Keywords: Fibroma, Pregnancy, In silico prediction, CYP17A1, Variability, Senegal.

1. Introduction

Uterine myomas, commonly known as fibroids, are benign tumors of the smooth muscle layer of the uterine wall and represent the most common solid pelvic neoplasms of reproductive age [1]. These lesions disrupt uterine functions and cause excessive uterine bleeding, anemia, defective embryo implantation, recurrent pregnancy loss, preterm labor, obstructed labor, pelvic discomfort, and urinary incontinence, and may mimic or mask malignant tumors [2,3]. Myomatous uterine pathology is common, affecting around 25% of women in active genital periods. This indicates the high probability of its interaction with pregnancy. This association, the frequency of which varies from 1% to 4%, will continue to grow, given the later onset of pregnancy and the incidence rate of myomas, which rises progressively with age [4]. The lower percentages reflect the results of older series, based on clinical examinations and intraoperative

Citation: Bineta Kénémé, Codou Diop, Ndèye Ka, *et al*. Mutational Penetrance of the CYP17A1 Gene in the Evolution of Uterine Fibroids Coexisting with Pregnancy in Senegalese Women. Journal of Genetics and Genetic Engineering. 2024;6(1): 01-15.

diagnosis at the time of Caesarean section. The highest rate is found in more recent series, where diagnoses are made during the course of pregnancy during repeated ultrasound examinations. De vivo et al, (2011) [5], in their study of pregnant women with myomas, found a significant increase in myoma size in a high percentage of patients in both the first and second half of pregnancy.

It is widely accepted that myomas develop rapidly during pregnancy due to the influence of hormonal stimulation and increased blood flow [6]. The genetic network of fibroids is represented by genes responsible for steroid hormone metabolism and their receptors, proliferation genes, cell contacts, angiogenesis and growth factors, including oncogenes, pro-inflammatory cytokines and their suppressors, microRNA genes, and methylation [7,8].The objective of this study is to evluate the evolution of the uterine fibroid and to understand the genetic link between fibroid and pregnancy in Senegalese women.

2. Materials and Methods

2.1. Samples

The study was carried out on 56 Senegalese patients, 20 of whom had fibroids associated with pregnancy (FUG) and 36 patients with fibroids only (FU). Uterine fibroids are benign tumours, so surgery is performed on tumour tissue only. These samples are sent directly to the Genomics Laboratory of the Population Genetics and Management Team at UCAD, where they are stored in tubes containing 96% alcohol for molecular analysis.

2.1.1. Dna Extraction, Pcr Amplification and Sequencing

Total DNA from each sample is extracted using the Zymo research kit and protocol. Cytochrome P450 c17A1 (steroid 17α-monooxygenase, 17α-hydroxylase, 17-alpha-hydroxylase, 17,20-lyase, 17,20-desmolase), a hydroxylase enzyme encoded in humans by the CYP17A1 gene, also known as CPT7; S17AH; P450C17, is studied. It possesses both 17α-hydroxylase and 17,20-lyase activities and is a key enzyme in the steroidogenic pathway that produces progestins, mineralocorticoids, glucocorticoids, androgens and estrogens. The CYP17A1 gene is located on chromosome 10 (10q24.q25) and contains eight exons and seven introns [9]. The 5' untranslated region (UTR) of CYP17A1 contains a polymorphism (T/C) 34 bp upstream of the translation start site [10] or 27 bp downstream of the transcription start site [11]. This change creates an additional SP1-type promoter site (CCACC instead of CCACT), which may lead to increased gene expression [11]. This polymorphism has been associated with increased estrogen and progesterone levels, which may be linked to the development of uterine fibroids [12].

Amplification of the promoter region (5'UTR) and exon 1 of the CYP17A1 gene was performed on 56 DNA extracts with a reaction volume of 25µl : 2 µl or 4 µl DNA extract, 18.4 µl pure PCR water or 16.4 µl for the 4 µl, 2.5 µl 10X buffer, 0.5 µl dNTP, 0.5 µl F primer (5'-TCCTGAGCCCAGATACCAT-3'), 0.5 µl R primer (5'-CCGCCCAGAGAAGTCCT-3'), 0.5 µl MgCl2, 0.1 µl Taq, 23 µl volume mix for 2 µl DNA or 21 µl volume mix for 4 µl DNA. Amplification conditions for the CYP17A1 gene are as follows: initial denaturation (94°C; 12min),35 cycles of denaturation (94°C; 30s), hybridization (60°C; 30min), elongation (72°C; 1min) and final elongation (72°C; 7min).

Sequencing involves determining the nucleotide sequence of a DNA fragment. Samples are suspended in distilled water and subjected to electrophoresis in ABI 3730xl sequencer (Appled Biosystems). The gel is read by automatic laser scanning to detect the various fluorochromes coupled to the 4 ddNTPs. The result of this scan is a chromatogram representing the succession of bases making up the DNA fragment.

2.2 Genetic Studies

2.2.1. Mutation Search

The chromatograms were submitted to Muation Surveyor v5.2 (www.softgenetics.com). This software uses patented technology to identify DNA variants from chromatograms with particular precision. The software detects the presence of homozygous and heterozygous (het) mutations, insertions (ins) and deletions (del) in comparison with the reference sequence of the human genome(NG_007955). The resulting sequences were then carefully checked, corrected and aligned using BioEdit v7.2.5 [13], with Clustal W algorithm [14].

2.2.2. In Silico Prediction of the Pathogenicity of Non-Synonymous Mutations

To determine deleterious, damaging, neutral or benign functional impact, non-synonymous variants (nsSNPs) are submitted to in Silico tools for pathogenicity prediction. The Polyphen2 (polymorphism and phenotype) server was used to predict the functional impact of amino acid substitutions on protein structure and function based on sequence characterization (http://genetics.bwh.harvard.edu/pph2/). The result

of the prediction was obtained in the form of a probability score that classifies variations as «probably damaging», «possibly damaging» and «benign» [15]. The UniProtKB P05093 protein accession ID and the position and name of the wild-type and variant amino acids of the selected nsSNPs were submitted as a query.

Meta-SNP is a random forest-based binary classifier to distinguish between disease-related Single Nucleotide Variants (SNVs) and non-synonymous polymorphic SNVs. Meta-SNP takes as input the output of the four predictors described above in the form of an eight-element feature vector composed of two groups of four elements each. The first group is the set of raw output scores from the variant predictions of PANTHER, PhD-SNP, SIFT and SNAP. In the event that one of the input methods did not return a prediction, Capriotti et al, (2013) [16] used the default threshold defined by the method to differentiate neutrals and non-neutrals in the Meta-SNP input (SNAP=0, SIFT=0.05, PhD-SNP=0.5, PANTHER =0.5).

2.2.3. In Silico Methods for Predicting the Influence of Mutations on Protein Stability

To determine the influence of non-synonymous variants on protein stability we subjected them to I-mutant2.0, iStable 2.0 and DUET : I-mtant2.0: is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes during point mutations. Predictions on the I-Mutant2.0 web page are made from either the protein structure or, more importantly, the protein sequence.

iStable 2.0: to evaluate the performance of the prediction model and compare it with other methods, Chen et al, (2020) [17] prepared an independent test set from data obtained from ProTherm (thermodynamic database for proteins and mutants) [18], containing 2869 point mutations in 81 proteins. Each point mutation in I-Mutant, PoPMuSiC and ProTherm contains five types of information: 1) the Protein Data Bank (PDB) ID, which contains information on the 3D structure of the proteins, 2) the site of the mutation position and residue with the native and mutant proteins, 3) the temperature used in the experiment, 4) the pH used in the experiment and 5) the change in free energy between the wild-type and mutant protein (ΔΔG or DDG). DUET: is an integrated computational approach to predict the effects of missense mutations on protein stability. DUET combines mCSM and SDM into a consensus prediction, consolidating the results of the separate methods into an optimized predictor using support vector machines (SVMs) trained with sequential minimal optimization [19]. The ΔΔG value is the difference between the Gibbs free energy of the mutated protein and that of the wild-type [20]. A ΔΔG value <0 indicates that the variation has a negative impact on protein stability. A ΔΔG value >0, on the other hand, suggests that the variation improves protein stability.

2.2.4. Relative Prediction of Solvent Accessibility and Secondary Structure

To predict the relative solvent accessibility and secondary structure of the CYP17A1 protein, we submitted its protein sequence to the NetSurfP-2.0 web server. NetsurfP-2.0: predicts solvent accessibility, secondary structure, disorders and phi/psi dihedral angles of amino acids in an amino acid sequence. The FASTA format of the CYP17A1 protein sequence was submitted as an input request to this server.

As output we first have a table combining the mutant's exposed or conserved assignment information, the relative accessibility area in percent, the absolute accessibility area in Angstrom, the mutant's position in the secondary structure (helix α, coil or Strand β), the percentage of disorder and the Phi and Psi dihedral angles (φ and ψ) and in figure the secondary structure with the mutated amino acids and disorder based on the thickness of the gray line [21].

2.2.5. 3d Structure of the Cyp17a1 Protein

To determine the impact of mutated amino acids on the dynamics of the protein compared with the reference and its interactions, and the structural damage involved, we submitted each non-synonymous variant to the Dynamut2 and Missense 3D servers. Dynamut2: is a web server that combines analysis methods using a range of features, including protein dynamics (NMA), wild-type residue environment, substitution propensities and contact potential scores, interatomic interactions [22] as well as the well-validated graph-based signature approach [23,24,25]. The protein destabilizing effect is marked by a ΔΔG (< 0.0 kcal/mol) and the stabilizing effect by a ΔΔG (> 0.0 kcal/mol). As input for the single mutation option, a protein structure in PDB format, the chain identifier where the mutation occurs and the point mutation defined as a chain comprising a one-letter-coded wild-type residue, residue position and one-letter code of the mutant residue are provided. For point mutations on the «Single Mutation» option, the predicted ΔΔG is displayed at the top along with user input details and the wild-type residue environment. All interatomic contacts calculated with Arpeggio are also displayed as an interactive viewer using the NGL viewer.

Missense 3D: is a web server that predicts structural changes introduced by an amino acid substitution and is applicable for analyzing both PDB coordinates and homology-predicted structures. Mutant and wild-type structures were analyzed to identify whether the structural consequence of the substitution should be detrimental in terms of the stability of the folded protein. Based on well-established principles of protein conformation and previous studies on the structural consequences of disease-associated substitutions, Yue et al., (2005); Al- Numair et al., (2013) and Bhattacharya et al., (2017) [26,27,28] considered 17 structural features.

2.2.6. Gene/Gene Interaction Analysis

The GeneMANIA Cytoscape application can be used to construct a weighted composite functional interaction network from a list of genes [29]. Each node represents a gene and its products. The application uses the GeneMANIA algorithm to find other genes and gene products most related to the original list and shows how they are linked [30]. The application provides access to most of the features of the GeneMANIA prediction server, while removing limitations on the length of the gene list and the maximum size of the resulting network [31].

2.2.7. Protein/Protein Interaction Analysis

STRING maps were used to describe the protein-protein interaction of CYP17A1. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database aims to collect, record and integrate all publicly available sources of information on protein-protein interactions, and to complement them with computational predictions. Its aim is to create a comprehensive and objective global network, including direct (physical) and indirect (functional) interactions [32].

3. Results

We performed PCR-sequencing analysis of CYP17A1 gene in 56 patients, 20 of whom had fibroids associated with pregnancy, and 36 had fibroids only.

3.1 Nature and Position of Mutations

Analysis of the chromatograms using Mutation Surveyor software revealed the presence of mutations both in the fibroid tissue of pregnant women and in the fibroid tissue of non-pregnant women (Figure 1).

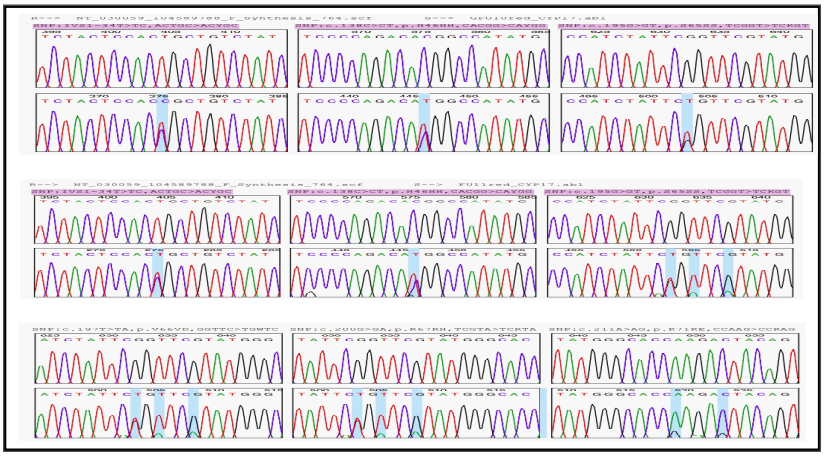


Figure 1. *Some SNPs of the CYP17A1 gene in FUG and FU tissues.*

A total of 123 mutations were found, including 99 mutations either in the intronic region or the 5'UTR and 21 in the exonic region, with 16 mutations that are non-synonymous, 3 synonymous mutations and 2 nonsense mutations, 1 deletion and 3 insertions. Of all 123 mutations, 24 (19.51%) were specific to patients with fibroids and pregnancy, 51 (41.46%) to fibroids only, and 48 (39.02%) to both conditions (Figure 2).

Of all the mutations found, 70 are new and 53 are listed in the dbSNP database, but most of these are deposited mutations, so no information is available to date on their clinical significance or the pathologies in which they are implicated. On the other hand, of the 16 non-synonymous mutations, 5 (p.Lys26Thr, p.Ser30Asn, p.Ser39Asn, p.Arg45Ile, p.Lys55Glu) are specific to fibroid tissue and pregnancy, and 11 (p. Val5Gly, p.Leu8Val, p.Tyr14Asn, p.Phe16Ile, p.Asn51Lys, p.Gln57His, p.Val66Asp, p.Arg67His, p.Lys71Glu, p.Thr72Ser, p.Gln80His) are specific to fibroid tissue only. The c.-34T>C polymorphism, located in the 5' promoter region 27bp downstream of the transcription start site, was found in both fibroids and pregnancy and fibroids without pregnancy.

The mutation frequencies show the high expression of c.138C>T (p.His46>His) with a frequency of 53.571%, c.-34T>C corresponding to the desired polymorphism with a frequency of 51.785% and c.195G>T (p.Ser65>Ser) with a frequency of 50%.

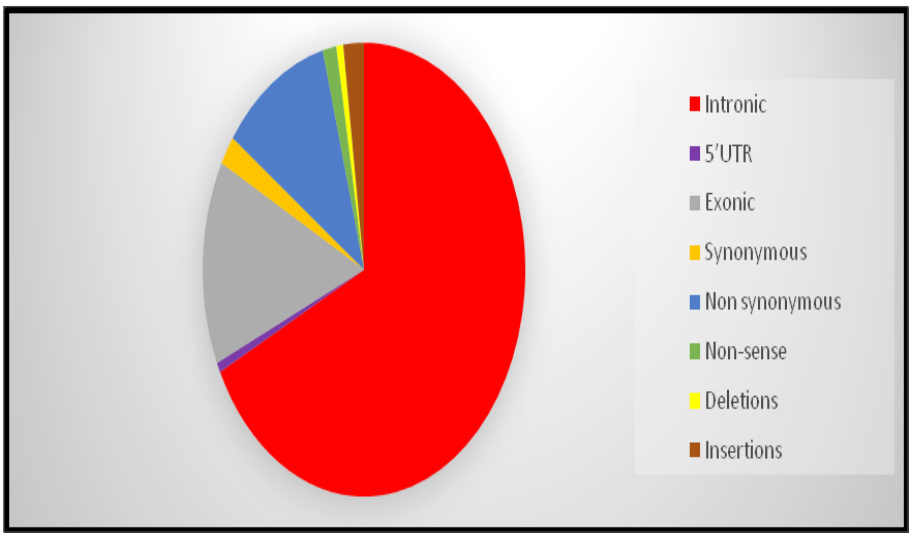


Figure 2. *Total number of mutations in the amplified gene region.*

3.2. Pathogenicity of Mutations

The 16 non-synonymous mutations were subjected to in Silico tools to determine their functional impact (deleterious, damaging or neutral). Of these 16 non-synonymous mutations, 5 (p.Val5Gly, p.Ser30Asn, p.Gln57His, p.Val66Asp and p.Arg67His) are predicted as probably damaging or possibly damaging (Polyphen2) and pathogenic by more than 3 Meta-SNP tools. The remaining 11 mutations are considered benign by Polyphen2 and neutral by Meta-SNP tools. Mutations predicted as pathogenic by more than 3 tools are only included in (Table 1).

Table 1. *Pathogenicity of non-synonymous mutations.*

		Meta-SNP				
Mutations	Polyphen 2	Panther (score)	PhD-SNP (score)	Sift (score)	SNAP (score)	Meta-SNP (score)
p.Val5Gly	Probabledommage (0.930)	Pathogen (0.599)	Neutral (0.391)	Neutral (0.090)	Pathogen (0.545)	Pathogen (0.614)
p.Ser30Asn	Probabledommage(0.996)	Pathogen (0.720)	Neutral (0.479)	Neutral (0.110)	Neutral (0.430)	Pathogen (0.590)
p.Gln57His	Probabledommage(1)	Pathogen (0.823)	Neutral (0.352)	Pathogen (0.020)	Pathogen (0.590)	Pathogen (0.667)
p.Val66Asp	Probabledommage(0.979)	Pathogen (0.672)	Pathogene (0.821)	Pathogen (0.000)	Pathogen (0.705)	Pathogen (0.793)
p.Arg67His	Probabledommage(0.978)	Disease (0.550)	Neutral (0.428)	Neutral (0.440)	Neutral (0.445)	Pathogen (0.612)

3.3. Prediction of the Influence of Non-Synonymous Mutations on Protein Stability

Analysis of the effect of non-synonymous mutations on protein stability shows that only 3 (p.Arg45Ile, p.Lys55Glu and p. Lys71Glu) of the 16 mutations are predicted by more than 3 in silico tools (I-Mutant, iStable, the 2 DUET sub-tools and DUET itself) as stabilizing the CYP17A1 protein, with positive Gibbs free energy difference values between wild-type and mutant, in contrast to those destabilizing the protein, where Gibbs energy differences are negative(Figure 3).

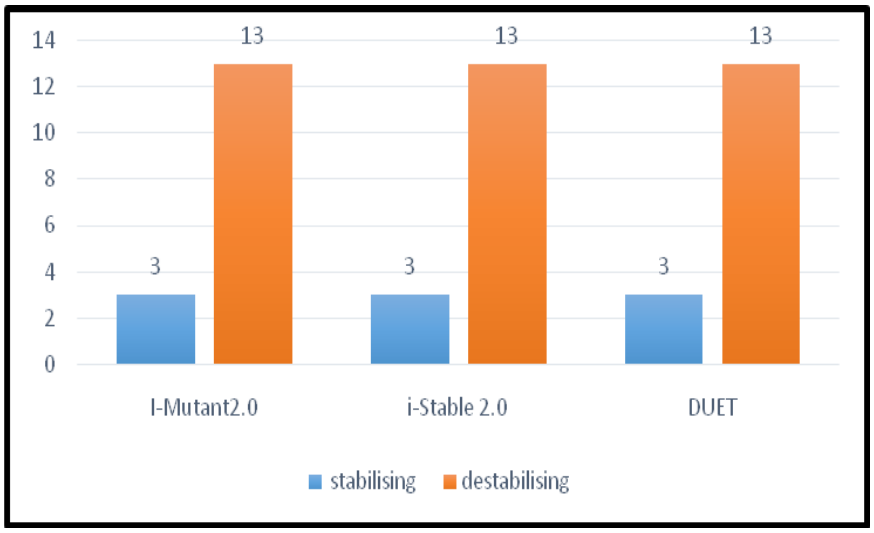


Figure 3. *In silico prediction of nsSNVs on protein stability.*

3.4. Solvent Accessibility, Disorder Prediction and Secondary Structure

Relative analysis of solvent accessibility surface area first shows us the presence of residue disorder based on grey line thickness (Figure 4) in 6 variants (p.Val5Gly, p.Leu8Val, p.Tyr14Asn, p.Phe16Ile, p.Lys26Thr, p.Ser30Asn) with their respective percentages (Table 2). Then we note significant relative percentages of the accessibility surface (31% to 67%) for the mutant amino acids designated as exposed (p.Val5Gly, p. Leu8Val, p.Tyr14Asn, p.Phe16Ile, p.Lys26Thr, p.Ser30Asn, p.Arg45Ile, p.Asn51Lys, p.Lys55Glu, p.Lys71Glu, p.Gln80His) compared with mutants designated as buried (6% to 22%). For secondary structure, only 1 exposed variant is located in the β-sheet (p.Lys71Glu), 1 variant (p.Val5Gly) is located in the Coil/Helix, 5 variants (p. Tyr14Asn, p.Phe16Ile, p.Lys26Thr, p.Ser30Asn and p.Arg45Ile) exposed are located in the Coil and 4 (p.Leu8Val, p.Asn51Lys, p.Lys55Glu and p.Gln80His) on the Helix.

Table 2. *Relative accessibility surface and disorder prediction.*

Mutations	Net SurfP-2.0				
	Assignment	RSA in %	ASA in Å	Structure secondaire/P disorder (%)	Phi Psi
p.Val5Gly	Exposed	67	52	Coil/α helix (11)	-63 -41
p.Leu8Val	Exposed	63	97	Helix/α helix (11)	-66 -42
p.Tyr14Asn	Exposed	47	69	Coil (18)	-73 -33
p.Phe16Ile	Exposed	62	115	Coil (12)	-84 -22
p.Lys26Thr	Exposed	68	95	Coil (7)	-100 132
p.Ser30Asn	Exposed	59	87	Coil/Turn(4)	-75 43
p.Ser39Asn	Buried	13	19	Coil (0)	-102 92
p.Arg45Ile	Exposed	31	57	Coil/Turn(1)	-81 -4
p.Asn51Lys	Exposed	47	96	Helix/α helix(0)	-66 -40
p.Lys55Glu	Exposed	44	77	Helix/α helix(0)	-65 -42
p.Gln57His	Buried	19	34	Helix/α helix(0)	-63 –40
p.Val66Asp	Buried	6	8	Strand/β-sheet (0)	-127 139
p.Arg67His	Buried	22	41	Strand/β-sheet(0)	-110 130
p.Lys71Glu	Exposed	31	54	Strand/β-sheet(0)	-110 145
p.Thr72Ser	Buried	20	24	Strand/β-sheet(0)	-86 136
p.Gln80His	Exposed	51	94	Helix/α (0)	-66 -39

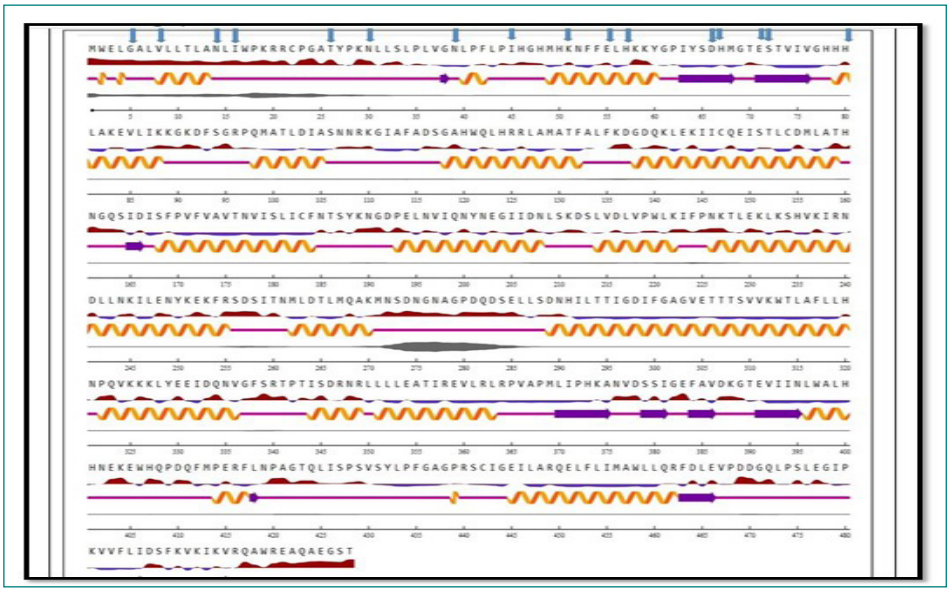


Figure 4. *Secondary structure of the CYP17A1 protein.*

NB : Surface accessibility: The red upward elevation indicates the exposed residue, while the sky-blue downward elevation indicates the residue buried in the protein structure. Secondary structure: The straight pink line is the coil, the orange spiral is the helix and the coloured arrow (indigo) is the strand. Disorder: Below the secondary structure prediction line is the thick greyish line indicating the probability of disorder linked to this residue. The wider the line, the greater the risk of disorder.

3.5. 3d Protein Structure

More than half of the variants (13) are predicted as destabilizing the CYP17A1 protein structure by the Dynamut2 server with negative Gibbs free energy difference values between wild-type and mutant amino acids. The 3 remaining variants (p.Tyr14Asn, p.Phe16Ile and p.Arg45Ile) are predicted to stabilize the protein. On the other hand, the Missense 3D server classifies 15 of the 16 non-synonymous mutations as presenting no damage to the protein structure (Figure 5). The p.Val66Asp variant is the only one classified as inducing structural damage to the tertiary structure of the CYP17A1 protein.

It is classified on the fourth (Buried hydrophilic introduced) and fifth (Buried charge introduced) characteristic of the 17 Missense 3D features. In other words, the substitution of Valine by Aspartic acid results in the replacement of the conserved hydrophobic residue by a hydrophilic one, and the neutral (uncharged) Valine residue is replaced by a charged one.

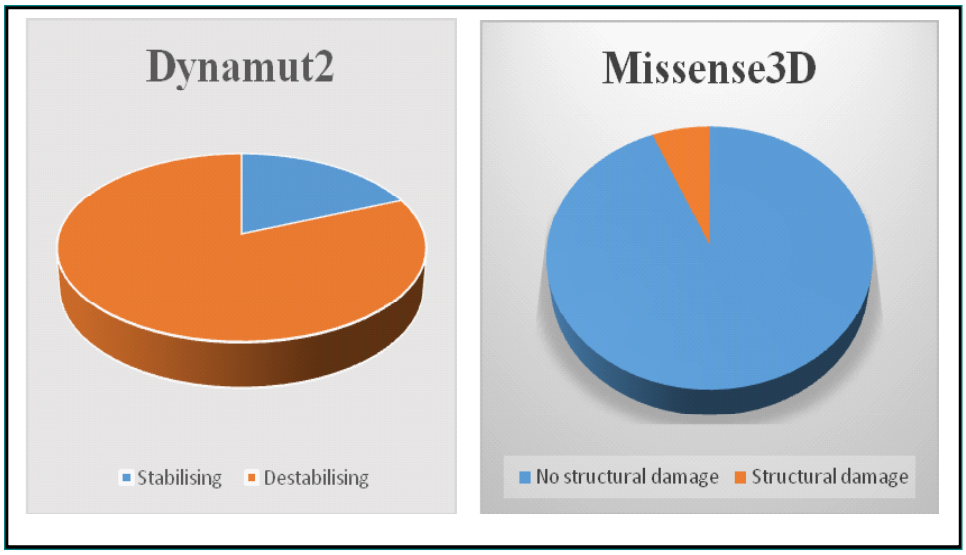
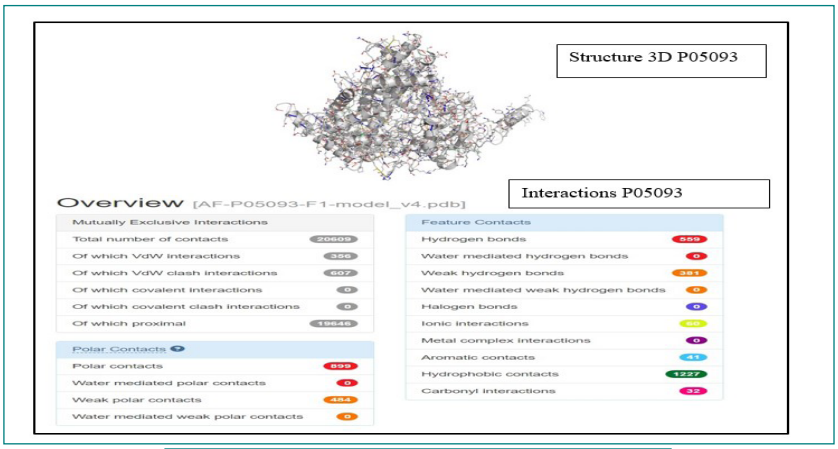


Figure 5. *Impact on CYP17A1 protein stability and structure.*

Analysis of the 3D structure of the mutant shows changes in binding compared to wild-type residues and to the reference (AF-P05093-F1-model_v4.pdb), determined by the protein's ARPEGGIO server. We note bond losses, the appearance of new bonds, conformational changes and substitutions by charged, hydrophobic or hydrophilic residues. These observations are shown in (Figure 6).



3D Structure and Interactions P05093

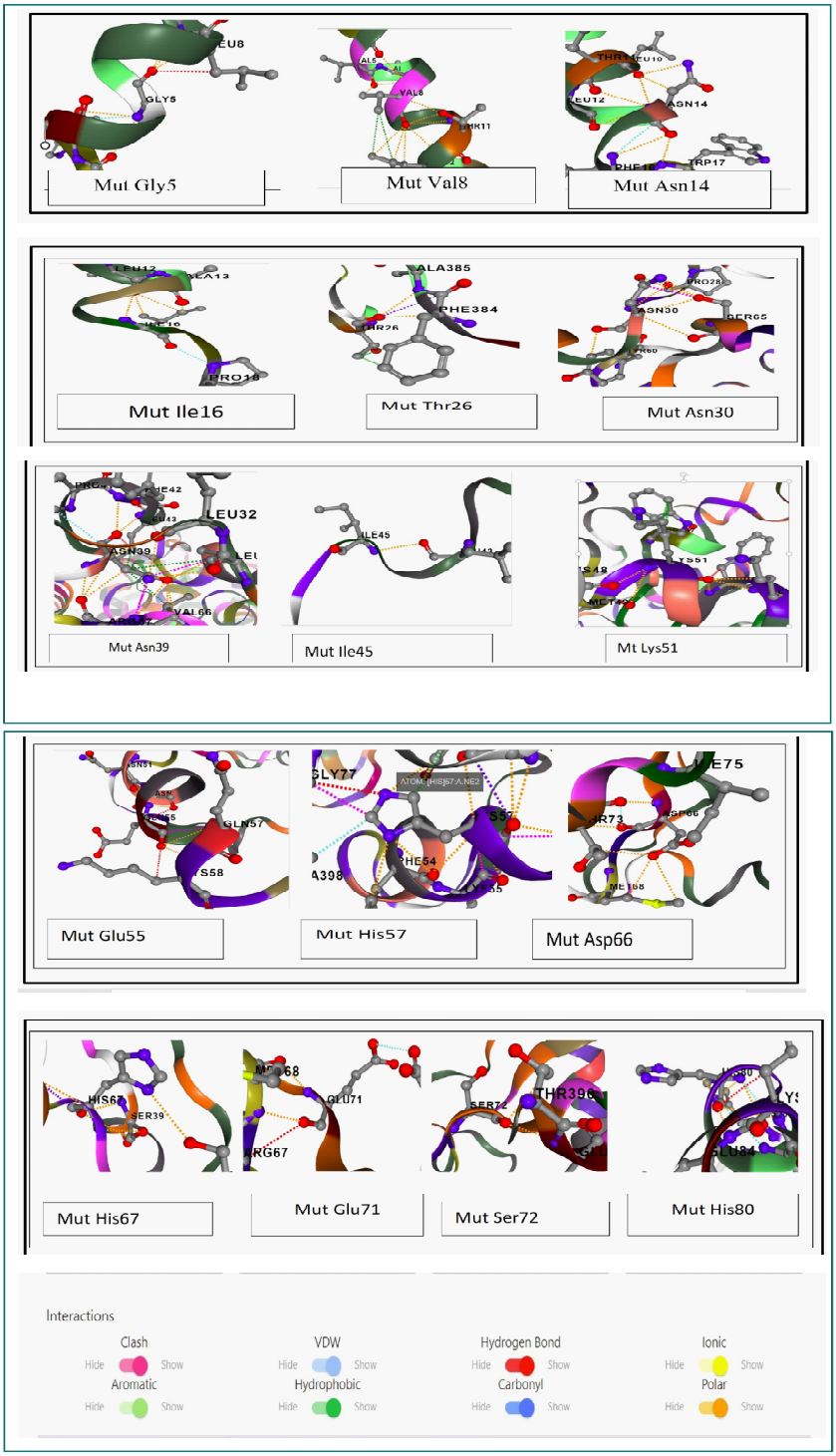


Figure 6. *3D protein structure*

3.6 Gene/Gene Interactions

Prediction results from the GeneMANIA server show that the CYP17A1 gene is linked physically and by co-expression to the HSD3B2, HSD3B1 and HSD17B3 genes, which also interact physically with each other, has protein domains with CYP21A2, POR, is linked to the CYP19A1 gene by physical and genetic interactions, and to the NFIC, SP3 and PBX genes by genetic links only. PTPA is weakly linked by physical interactions with CYP17A1.

On the other hand, CYP4B1, AVPRA1, CDH3, RPS4Y1, AKT2, CCN3, ID3, STAR, CYP11A1 and CPB1 are linked by co-expression with CYP17A1 (Figure 7).

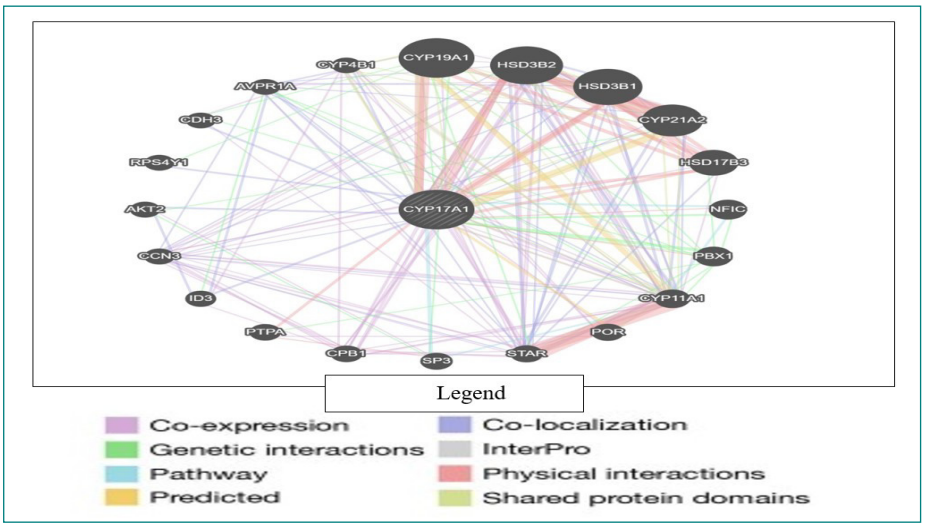


Figure 7. *Gene/gene interactions.*

3.7 Protein/protein interactions

The CYP17A1 protein/protein interaction network using STRING maps shows us a functional association of the CYP17A1 protein with other proteins. The protein shows strong direct functional associations (co-expression, co-occurrence and homology) with the following proteins: HSD17B3, HSD17B2, CYP19A1, HSD3B1, HSD3B2, AKR1C3. SRD5A1, SRD5A2, CYB5A, CYP5B are weakly linked (co-expression and homology) to the CYP17A1 protein, but nevertheless share biological functions. These observations are presented on the(Figure 8)

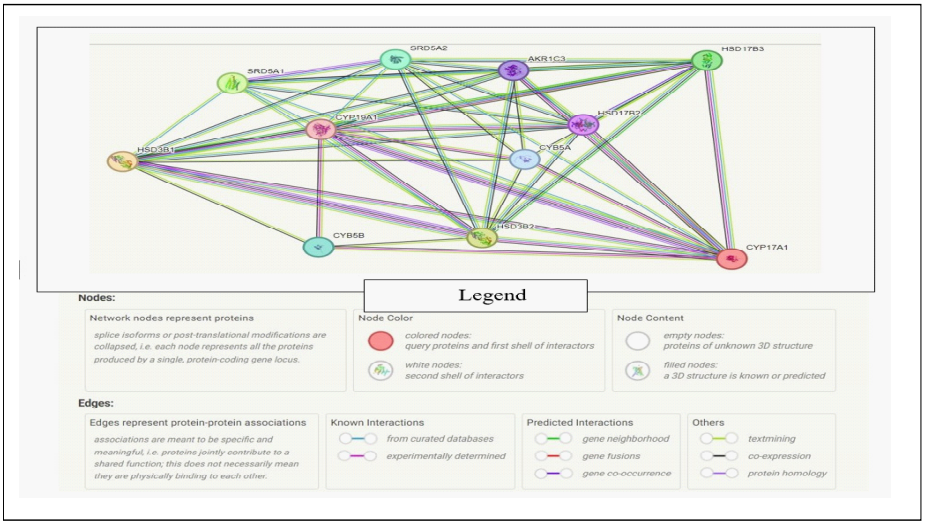


Figure 8. *Protein/protein interactions.*

4. Discussion

The aim of the present study was to identify the CYP17A1 gene mutations involved in uterine fibroids in pregnant Senegalese women, compared with women with fibroids who were not pregnant, and the functional impact of these mutations on protein stability. A total of 56 patients were studied. The cause of fibroids is unknown. It appears that elevated levels of estrogen and possibly progesterone (female hormones) stimulate their growth. According to Mutch and Biest, (2023) [33] fibroids can develop during pregnancy (when levels of these hormones rise, and they tend to disappear at menopause, when these levels drop drastically).

In line with the biological hypothesis that polymorphisms in genes involved in sex hormone

pathways could influence estrogen levels [34], we selected one of the genes affecting estradiol metabolism, cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1). In the present study, 123 mutations were found, showing that the CYP17A1 gene is highly polymorphic. Mutations found in both fibroid tissue and pregnancy, as well as in fibroid tissue alone, suggest that the CYP17A1 gene is involved in the incidence of uterine fibroids.

Although the triggers leading to the development of uterine leiomyomas are unknown, there is considerable evidence that sex steroid hormones are important factors in tumour growth [35,36]. Allelic variants in genes involved in steroidogenesis or steroid metabolism may contribute to susceptibility to uterine leiomyomas. More than half of the intronic mutations are common to both fibroid tissue in pregnant women and fibroid tissue alone.

Although they generally do not directly affect the amino acid sequence of a protein, they can have indirect effects on gene regulation or the splicing process. According to Daw et al, (2011) [37], intronic mutations in the CYP17A1 gene would cause cryptic splicing in 17α-hydroxylase (17OHD) deficiency.

Ideally, direct demonstration of the functional consequences of intronic CYP17A1 mutations requires analysis of gonadal or adrenal tissue to confirm this hypothesis [38,39]. Mutation frequencies show significant expression of the c.138C>T variant (p.His46>His) with a frequency of 53.571%, followed by the c.-34T>C variant (SNP Ref: rs743572) corresponding to the polymorphism we were looking for, with a frequency of 51.785%, and finally the c.195G>T variant (p.Ser65>Ser) with a frequency of 50%. The c.-34T>C variant was present in 17 non-pregnant women with fibroids and in 12 pregnant women with fibroids.

This shows that the c-34T>C polymorphism could influence the onset of uterine fibroids and its evolution during pregnancy by resulting in overexpression of the gene in the environment. Some relevant studies are consistent with the hypothesis that the TC variant, by altering gene function in one way or another, at least alters hormone concentrations, estrogen levels in premenopausal women.

The dominant sites of steroid hormone production in premenopausal and postmenopausal women are the ovaries and adrenal glands respectively, and it has been suggested that CYP17A1 expression may differ between these sites [40]. One report showed that in African women, homozygous carriers of CYP17A1 (TC) were at high risk of developing uterine leiomyoma, whereas in Caucasian women, the CYP17A1 genotype did not contribute to the etiology of uterine leiomyoma [41].

Amant et al, (2004) hypothesized that higher estrogen levels in African women homozygous for the CYP17A1 allele (TC) expose the myometrium to a stronger stimulatory effect, which could, in the long term, lead to spontaneous mutations and uncontrolled growth; an important feature of uterine fibroids. The fetus and placenta produce and secrete steroids and peptides into the maternal circulation, stimulating the production of maternal hormones [42,43].

Progesterone, estrogens, androgens and glucocorticoids are involved in pregnancy from implantation to delivery. Their biosynthesis and metabolism involve complex pathways due to the interaction between fetus, placenta and mother [44]. In the study, synonymous mutations (p.His46His, p.Ser65Ser and p.Lys71Lys) appear to have no effect on gene expression or protein function, as the protein produced is identical. But this is called into question because it has been shown that certain genetic diseases are linked to the presence of a synonymous mutation in the genome.

It has also been shown that these mutations act not on the quality of the proteins produced (which are identical), but on their quantity [45]. The c.138C>T variant (p.His46His) is benign or probably benign with regard to pathologies linked to the MTOR genes, EGLN1 involved in the predisposition of hereditary cancer syndromes, PRSS1 and TRB for hereditary pancreatitis, COL6A2 for myosclerosis.

The c.195G>T variant (p.Ser65Ser) is found only on the CYP17A1 gene, where it is benign in steroid 17α-monooxygenase deficiency activity. The c.213G>A variant (p.Lys71Lys) is found in the RAD50 and TSC2 genes, which are involved in predisposition to hereditary cancer syndromes, and CYBA in chronic granulomatous disease, where it has a benign effect.

With regard to non-synonymous mutations, the five variants (p.Val5Gly, p.Ser30Asn, p.Gln57His, p.Val66Asp and p.Arg67His), of which only one (p.Ser30Asn) is specific to fibroid tissue and pregnancy, predicted as probably damaging and pathogenic by all in silico prediction software, could alter normal protein synthesis and thus cause overexpression of the gene in the environment. This overexpression of the gene, which will be translated into elevated steroid levels, could induce tumor cell proliferation in women

with fibroids only without being pregnant, and fibroid cell growth throughout pregnancy. The c.14T>G (p.Val5Gly) mutation concerns the 5th amino acid of exon 1 of the gene, it could lead to a molecular defect in CYP17A1 protein synthesis.

The c.76A>T (p.Lys26*) mutation results in a truncated CYP17A1 protein, which could cause premature loss of function, with the 17α-hydroxylase and 17,20-lyase functions of the said gene being deficient. This deficiency is a condition that affects the functioning of certain hormone-producing glands called the adrenal glands. The pathogenic c.194C>A (p.65Ser*) mutation in the MSH6 gene, located in coding exon 1, results from a C-to-A substitution at nucleotide position 194.

This changes the amino acid of a serine into a stop codon in coding exon 1. This alteration should lead to loss of function through premature protein truncation or nonsense mRNA decay. As such, this alteration is interpreted as a pathogenic mutation [46]. Nonsynonymous nucleotide substitutions (nsSNPs) are of particular interest, as they can disrupt function by interfering with protein stability and/or partner interactions. Such mutations can be selectively advantageous in evolution, or they can cause a change in stability often leading to dysfunction and disease.

Thus, predicting the impact of mutations in proteins is of major importance for understanding the function, not only of molecules and cells, but of the whole organism [47]. The three mutations (p.Arg45Ile, p.Lys55Glu and p.Lys71Glu) predicted to stabilize the protein by in silico tools appear to have no effect on protein stability. This could be explained by the fact that the location and type of mutated amino acid are in a less critical region of the protein, thus presenting a more moderate effect.

The stability of a protein refers to its ability to retain its shape and structure under varying conditions [48]. This is crucial for its proper functioning and efficacy. Thus, even with the presence of these three variants, the CYP17A1 protein could retain its structural form and resist mutant amino acid changes that could alter its function. But this remains to be seen, as the properties of the mutant amino acid may differ from those of the wild-type.

In contrast to the stabilizing ones, the thirteen variants predicted to have protein instabilizing effects could impact on the degradation of the protein to be synthesized. If wild-type amino acids are essential for protein function, then these mutations may alter the protein's ability to perform its normal function. With regard to relative surface accessibility, conserved variants appear not to induce effects in the management of the biological system, including stability, folding or both, unlike exposed variants.

The latter with a high accessibility surface are located on the protein surface which could lead to losses of interaction and structural alteration. β-sheets have a much lower frequency of occurrence than α-helices. The exposed variant located in the β-sheet (p.Lys71Glu) could affect the stability of the protein structure and lead to the incidence of the pathology. Exposed variants (p.Leu8Val, p.Asn51Lys, p.Lys55Glu and p.Glu80His) of the α-helix could lead to instability in structure, function and ability to interact with other molecules. Polar charge is a factor in stability.

An α-helix is generally positively charged at one end and negatively charged at the other, which can destabilize the protein's structure. Mutations located in the coil (p.Tyr14Asn, p.Phe16Ile, p.Lys26Thr, p.Ser30Asn and p.Arg45Ile) could have negative effects on protein function and structure. The protein's 3D structure indicates that the mutations have caused structural damage to the protein, which may affect its biological function, its membrane transport capacity due to the hydrophobic mutants (Valine, Leucine, Phenylalanine, Isoleucine and Glutamine), its molecular cohesion function due to ionic mutants creating a positive (Arginine, Lysine and Histidine) or negative (Lysine, Asparagine and Glutamic acid) opposite charge, and the alteration of these electron sites due to mutants with polar characteristics (Tyrosine, Asparagine, Threonine, Serine and Histidine).

Significant local conformational changes at the membrane interface accompany the process of protein insertion into the membrane. On the basis of structural analysis, the overall protein structure and topology of CYP17A1 are quite reserved during membrane binding. Local conformational changes, mainly at the membrane-protein interface, are probably due to the interaction of CYP17A1 with the membrane and may have a further impact on the opening and closing of different tunnels for efficient recruitment of lipophilic substrates from the membrane to the enzyme's buried active site.

In general, the predominant hydrogen-bonding interactions with lipid molecules were either via basic residues (Lysine and Arginine) and polar residues (Serine, Threonine, Glutamine and Asparagine), or via the backbones of hydrophobic residues (in

particular Leucine and Phenylalanine) to a lesser extent. In addition to electrostatic interactions, the globular domain of CYP17A1 came into contact with the membrane mainly via hydrophobic anchoring.

Overall, many of the specific interactions identified to anchor the protein in the membrane environment were suggested to be closely linked to determining the well-defined orientations of CYP17A1 relative to the plane of the membrane. It is hypothesized that these hydrogen bonds as well as hydrophobic interactions mediate the interactions of the globular domain of CYP17A1 with the membrane and may have an impact on the catalytic activity of CYP17A1 in the lipid bilayer. [49].

The interaction of the enzyme with the membrane, which could affect the structural and dynamic characteristics of the enzyme. Steroidogenic enzymes fall into two groups: cytochrome P450 enzymes and hydroxysteroid dehydrogenases. A cytochrome P450 can be either type 1 (in the mitochondria) or type 2 (in the endoplasmic reticulum), and a hydroxy steroid dehydrogenase can be either type 1 (in the mitochondria) or type 2 (in the endoplasmic reticulum) may belong to the aldo keto reductase or short-chain dehydrogenase/reductase family.

The activities of these enzymes are modulated by post-translational modifications and cofactors, in particular electron-donating redox partners. Elucidation of the precise roles of these different enzymes and cofactors has been greatly facilitated by the identification of the genetic basis of rare diseases of steroidogenesis [50]. Of the 56 individuals, 9 show no variability in exon1 of the gene.

This could be explained by the fact that other genes are involved in fibroma occurrence, hence the importance of analyzing gene and protein interactions with the GeneMANIA and STRING servers. The CYP17A1 gene shares almost identical functions with HSD3B2 (hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2), HSD3B1 (hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1) and HSD17B3 (Hydroxysteroid 17-Beta Dehydrogenase 3) also belong to CYP450, as does CYP21A2 (cytochrome P450 family 21 superfamily A member 2), CYP19A1 (cytochrome P450 family 19 subfamily A member 1), CYP4B1 (cytochrome P450 family 4 subfamily B member 1) and CYP11A1 (cytochrome P450 family 11 subfamily A member 1), all of which are involved in the sex hormone biosynthetic pathway (also known as steroidogenesis), an enzymatic cascade by which cholesterol is converted into biologically active steroid hormones. Variants destabilizing normal CYP17A1 protein synthesis include (p. Lys26Thr, p.Ser30Asn and p.Ser39Asn) for fibroid tissue and pregnancy and (p.Val5Gly, p.Leu8Val, p.Tyr14Asn, p.Phe16Ile, p.Asn51Lys, p.Gln57His, p.Val66Asp, p.Arg67His, p.Thr72Ser and p. Gln80His) for fibroid tissue alone could cause the same effects at both gene and protein levels, leading to the incidence of the pathology studied, since gene interactions often occur when mutations affect genes involved in the same biosynthetic, regulatory or developmental pathway.

Gene interaction analysis helps us to understand how genes work together to perform their functions in cellular physiology. Co-expression occurs when several genes are expressed under similar conditions. In other words, these genes show similar expression patterns in different samples. They highlight genes that are controlled by the same transcriptional regulatory program, functionally linked, members of the same gene regulatory network [51].

Strongly interconnected modules or subgraphs in gene co-expression networks (GCNs) often correspond to groups of genes with a similar function or participating in a common biological process. Concerning protein/protein interactions (PPIs) with the STRING server, the predictions show proteins in gene/gene interactions and we note that they share the same functions, i.e. the sex steroid biosynthesis pathway. A protein-protein interaction occurs when two or more proteins bind to each other, usually in order to perform their biological function [52].

These interactions are at the heart of the interatomic system of the living cell, and play an essential role in many genetic functions. Interactions between proteins are important for most genetic functions. For example, a signal from outside the cell is transmitted inside by protein-protein interactions of the molecules making up the signal. This mode of operation, known as «signal transduction», plays a fundamental role in most biological processes and in many diseases, such as cancer. Proteins may interact for long periods to form a piece of protein complex, one protein may transport another protein (e.g. from cytoplasm to nucleus or vice versa in the case of nuclear pore importins), or one protein may interact briefly with another, only to modify it [53].

Protein-protein interactions (PPIs) play a fundamental role at all levels of the cell, whether in metabolism, signalling, cell proliferation, intercellular communication or the maintenance of membrane

architecture. All these biological processes and functions involve a multitude of proteins acting in concert in complex, interconnected systems [54]. The p.Val66Asp variant, a conserved neutral hydrophobic residue replaced by a charged hydrophilic residue, could play a role in tumour proliferation due to its role in energy metabolism, as well as in their growth during pregnancy via increased gene expression. Aspartic acid (D-Asp) is involved in energy metabolism by contributing to the production of ATP, the main source of cellular energy [55]. D-Asp plays an important role in the biosynthesis and/or secretion of hormones in endocrine glands, modulates steroidogenesis in the adrenal gland and regulates testosterone production [56, 57, 58].

5. Conclusion

These results demonstrate the involvement of the CYP17A1 gene in the incidence of uterine fibroids in pregnant women in Senegal. The presence of the mutation in the promoter region of the gene (c.-34T>C) in the patients seems to confirm the hypothesis of Amant et al, (2004) that higher estrogen levels in African women homozygous for the CYP17A1 allele (TC) expose the myometrium to a stronger stimulatory effect, which could, in the long term, lead to spontaneous mutations and uncontrolled growth; an important feature of uterine fibroids.

The non-synonymous variants (p.Lys26Thr, p.Ser30Asn and p.Ser39Asn) for fibroid tissue and pregnancy and (p.Val5Gly, p.Leu8Val, p.Tyr14Asn, p.Phe16Ile, p.Asn51Lys, p.Gln57His, p.Val66Asp, p.Arg67His, p.Thr72Ser and p. Gln80His) for fibroid tissue alone induce a change in the enzyme's biological function by inhibiting progesterone metabolism by the CYP17A1 enzyme and thus overproducing the latter, on which steroid-mediated maintenance of pregnancy and fibroid growth depend.

Hydrophobic and ionic mutants affect the process of protein insertion into the membrane, while mutants with polar characteristics impact electron sites. Other genes and their interacting proteins with the same biological functions as CYP17A1 may be involved in the incidence of uterine fibroids in both pregnant and non-pregnant women.

6. References

1. MCWILLIAMS, Michelle M. and CHENNATHUKUZHI, Vargheese M. Recent advances in uterine fibroid etiology. In: Seminars in reproductive medicine. Thieme Medical Publishers, 2017. p. 181-189.
2. Baird DD, Dunson DB. Why is parity protective for uterine fibroids? Epidemiology. 2003 Mar ;14(2): 247-50.
3. Catherino WH, Parrott E, Segars J. Proceedings from the national institute of child health and human development conference on the uterine fibroid research update workshop. Fertil Steril 2011;1:9-12. Google ScholarCrossrefWorldCat
4. Lambling -A. Chauveaud, H. Fernandez. Fibroma and pregnancy. EMC-Gynécologie Obstétrique 1 (2004) 127-135.
5. De Vivo, A, Mancuso, A, Giacobbe, A, et al. Uterine myomas during pregnancy: a longitudinal sonographic study. Ultrasound Obstet Gynecol 2011;37:361-365.
6. Wallach EE, Vlahos NF. Uterine myomas: an overview of development, clinical features, and management. Obstet Gynecol 2004; 104: 393-406.
7. Kim J, Sefton EC. The role of progesterone signaling in the pathogenesis of uterine leiomyoma. Mol Cell Endocrinol. 2012;358:223-231.
8. Bulun S. Uterine fibromas. N Engl J Med 2013;369:14.
9. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17alpha (CYP17) gene polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Res 1998;58:585 -587.
10. Kadonaga JT, Jones KA, Tijan R. Promoter-specific activation of RNA polymerase II transcription by Sp1. Trends Biochem Sci 1986 ; 11 :20-3.
11. Catherino WH, Eltoukhi HM, Al-Hendy A. Racial and ethnic differences in the pathogenesis and clinical manifestations of uterine leiomyoma. Semin Reprod Med 2013;31:370 -379.
12. Kristensen VN, Borresen-Dale AL. Molecular epidemiology of breast cancer: genetic variation in steroid hormone metabolism. Mutat Res 2000 ;462 (2-3) :323-33.
13. Hall, T. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95-98.
14. Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2003). Multiple sequence alignment using ClustalW and ClustalX. Current protocols in bioinformatics, 1, 2-3.
15. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248-9.

16. Capriotti, E., Altman, R. B., & Bromberg, Y. (2013). Collective judgment predicts disease associated single nucleotide variants. BMC Genomics.

17. Chen CW, Lin MH, Liao CC, Chang HP, Chu YW. iStable 2.0: Prediction of protein thermal stability changes by integrating various feature modules. Comput Struct Biotechnol J. 2020 Mar 6;18:622-630. doi: 10.1016/j.csbj.2020.02.021. PMID: 32226595; PMCID: PMC7090336.

18. Bava KA, Gromiha MM, Uedaira H, Kitajima K, Sarai A. (2004). ProTherm, version 4.0: thermodynamic database for proteins and mutants. Nucleic Acids Res. 32, D120-D121.

19. Shevade,S.K., Keerthi,S.S., Bhattacharyya,C. and Murthy,K.R.K. (2012) Improvements to the SMO algorithm for SVM regression. IEEE Trans. Neural Netw. 11, 1188-1193.

20. Elkhattabi L, Morjane I, Charoute H, Amghar S, Bouafi H, Elkarhat Z, et al. In silico analysis of coding/noncoding SNPs of human RETN gene and characterization of their impact on resistin stability and structure. J Diabetes Res. 2019; 2019:4951627. doi: 10.1155/2019/4951627.

21. Petersen B, Petersen TN, Andersen P, Nielsen M, Lundegaard C. A generic method for assignment of reliability scores applied to solvent accessibility predictions. BMC Struct Biol. 2009 ; 9:51. doi : 10.1186/1472-6807-9-51.

22. Jubb,H.C., Higueruelo,A.P., Ochoa-Montano,B., Pitt,W.R., Ascher,D.B. and Blundell,T.L. (2017) Arpeggio: A web server to calculate and visualize interatomic interactions in protein structures. J. Mol. Biol. 429, 365-371.

23. Pires,D.E., Ascher,D.B. and Blundell,T.L. (2014) mCSM: predicting the effects of mutations in proteins using graph-based signatures. Bioinformatics, 30, 335-342.

24. Pires,D.E., Blundell,T.L. and Ascher,D.B. (2016) mCSM-lig: quantifying the effects of mutations on protein-small molecule affinity in genetic diseases and the emergence of drug resistance. Sci. Rep. 6, 29575.

25. Carlos H.M. Rodrigues, Douglas E.V. Pires, and David B. Ascher DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability W350-W355 Nucleic Acids Research, 2018, Vol. 46, Web Server issue Published online April 30, 2018 doi: 10.1093/nar/gky300.

26. P. Yue, Z. Li, J. Moult, Loss of protein structure stability as a major causative factor in monogenic disease, J. Mol. Biol. 353 (2005) 459-473. N.S.

27. Al-Numair, A.C. Martin, The SAAP pipeline and database: tools to analyze the impact and predict the pathogenicity of mutations, BMC Genomics 14 (2013) S4.

28. R. Bhattacharya, P.W. Rose, S.K. Burley, A. Prlić, Impact of genetic variation on protein three-dimensional structure and function, PLoS One 12 (2017), e0171355.

29. Shannon P, Markiel A, Ozier O, et al: Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11): 2498-2504. PubMed Abstract | Publisher Full Text | Free Full Text.

30. Mostafavi S, Morris Q: Fast integration of heterogeneous data sources for predicting gene function with limited annotation. Bioinformatics. 2010 ; 26(14) : 1759-1765. PubMed Abstract | Publisher Full Text | Free Full Text

31. Zuberi K, Franz M, Rodriguez H, et al: GeneMANIA prediction server 2013 update. Nucleic Acids Res. 2013; 41(Web Server issue): W115-W122. PubMed Abstract | Publisher Full Text | Free Full Text

32. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019 Jan 8;47(D1):D607-D613. doi: 10.1093/nar/gky1131. PMID: 30476243; PMCID: PMC6323986.

33. David G. Mutch and Scott W. Biest , Uterine fibromas (Leiomyomas; Myomas) MD, Washington University School of Medicine Verified/Revised May 2023.

34. Méar L, Herr M, Fauconnier A, et al. Polymorphisms and endometriosis: a systematic review and meta-analyses. Hum Reprod Update 2020; 26(1): 73-102.

35. Schwartz SM. Epidemiology of uterine leiomyomas. Clin Obst Gynecol 2001;44:316-26.

36. Wallach EE, Vlahos NF. Uterine myomas: an overview of development, clinical features, and management. Obstet Gynecol 2004;104:393-406.

37. Daw-Yang Hwang1 , Chi-Chih Hung1 , Felix G. Riepe2 , Richard J. Auchus3 , Alexandra E. Kulle2 , Paul-Martin Holterhus2 , Mei-Chyn Chao4,5, Mei-Chuan Kuo1,6, Shang-Jyh Hwang1,6*, Hung-Chun Chen1,6 CYP17A1 Intron Mutation Causing Cryptic Splicing in 17a-Hydroxylase Deficiency PLoS ONE | www.plosone.org September 2011 | Volume 6 | Issue 9 | e25492.

38. Suzuki Y, Nagashima T, Nomura Y, Onigata K, Nagashima K, et al. (1998) A new compound heterozygous mutation (W17X, 436+5G -.T) in

the cytochrome P450c17 gene causes 17 alpha-hydroxylase/17,20-lyase deficiency. J Clin Endocrinol Metab 83(1): 199-202.

39. Biason-Lauber A, Kempken B, Werder E, Forest MG, Einaudi S, et al. (2000) 17alpha-hydroxylase/17,20-lyase deficiency as a model to study enzymatic activity regulation: role of phosphorylation. J Clin Endocrinol Metab 85(3): 1226-1231.
40. Haiman CA, Hankinson SE, Colditz GA, et al. A polymorphism in CYP17 and endometrial cancer risk. Cancer Res 2001;61:3955-60.
41. Amant F, Dorfling CM, de Brabanter J, Vandewalle J, Vergote I, Lindeque BG, et al. A possible role of the cytochrome P450c17alpha gene (CYP17A1) polymorphism in the pathobiology of uterine leiomyomas from black South African women: a pilot study. Acta Obstet Gynecol Scand 2004;83:234-9
42. Yen SS. Endocrine regulation of metabolic homeostasis during pregnancy. Clin Obstet Gynecol 1973;16:130-47.
43. Tal R, Taylor HS, Burney RO, Mooney SB, Giudice LC. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, et al, editors. Endocrinology of pregnancy. South Dartmouth (MA): Endotext; 2000.
44. Morel Y, Roucher F, Plotton I, Simard J, Coll MD. 3beta-hydroxysteroid dehydrogenase deficiency. In: New MI, editor. Genetic steroid disorders. Elsevier; 2013. p. 99-110.
45. Shen, X., Song, S., Li, C., & Zhang, J. (2022). Synonymous mutations in representative yeast genes are mostly strongly non-neutral. Nature, 606, 725-731. http://doi.org/10.1038/s41586-022-04823-w (Original work published jun).
46. Devlin LA, Graham CA, Price JH, Morrison PJ. Germline MSH6 mutations are more prevalent in endometrial cancer patient cohorts than hereditary non polyposis colorectal cancer cohorts. The Ulster Medical Journal. 2008 Jan;77(1):25-30. PMID: 18269114; PMCID: PMC2397009.
47. Fersht,A.R. (1987) Dissection of the structure and activity of the tyrosyl-trna synthetase by site-directed mutagenesis. Biochemistry, 26, 8031-8037.
48. David Gnutt, Stepan Timr, Jonas Ahlers, Benedikt König, Emily Manderfeld, Matthias Heyden, Fabio Sterpone, Simon Ebbinghaus Stability Effect of Quinary Interactions Reversed by Single Point Mutations Journal of American Chemical Society - February 2019 DOI: 10.1021/jacs.8b13025
49. V. Cojocaru, P.J. Winn, R.C. Wade, The ins and outs of cytochrome P450s, Biochim. 699 Biophys. Acta 1770 (2007) 390-401
50. Y.-L. Cui, et al, Structural features and dynamic investigations of the membrane-bound cytochrome P450 17A1, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamem.2015.05.017.
51. Joshua M Stuart, Eran Segal, Daphne Koller and Stuart K Kim, «A gene coexpression network for global discovery of conserved genetic modules», Science, vol. 302, no. 5643, 2003, p. 24955 (PMID 12934013, DOI 10.1126/science.1087447, Bibcode 2003Sci...302..249S)
52. Matthew T Weirauch, «Gene coexpression networks for the analysis of DNA microarray data», Applied Statistics for Network Biology: Methods in Systems Biology, 2011.
53. Robin V, Bodein A, Scott-Boyer MP, Leclercq M, Périn O, Droit A. Overview of methods for characterization and visualization of a protein-protein interaction network in a multi-omics integration context [archive]. Front Mol Biosci. 2022 Sep 8;9:962799. DOI 10.3389/fmolb.2022.962799. PMID 36158572 [archive]; PMCID: PMC9494275.
54. Benoît Béganton, Etienne Coyaud, Alain Mangé, Jérôme Solassol Novel approaches to the study of protein-protein interactions medicine/sciences 2019; 35: 223-31.
55. John D. Fernstrom, Madelyn H. Fernstrom, Tyrosine, Phenylalanine, and Catecholamine Synthesis and Function in the Brain123,The Journal of Nutrition, Volume 137, Issue 6,2007,Pages1539S1547S,ISSN 00223166,https://doi.org/10.1093/jn/137.6.1539S. (https://www.sciencedirect.com/science/article/pii/S0022316622092720).
56. D'Aniello A, Di Cosmo A, Di Cristo C, Annunziato L, Petrucelli L, Fisher G. Involvement of D-aspartic acid in testosterone synthesis in rat testes. Vie Sci. 1996; 59 :97-104. [PubMed] [Google Scholar].
57. D'Aniello G, Tolino A, D'aniello A, Errico F, Fisher GH, Di Fiore MM. The role of d-aspartic acid and N-methyl-d-aspartic acid in the regulation of prolactin-1 release. Endocrinology. 2000; 141 :3862-3870 [PubMed] [Google Scholar].
58. Wang H, Wolosker H, Pevsner J, Snyder S, Selkoe D. Regulation of the rat magnocellular neurosecretory system by D-aspartate: evidence for a biological role(s) of a naturally occurring free D-amino acid in mammals. J Endocrinol. 2000; 167 :247-252. [PubMed] [Google Scholar.