

# Uterine Fibroid Diagnosed During Pregnancy: A Case Report

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## Abstract

Uterine fibroids arise from the smooth muscle cells of the myometrium and contain large amounts of extracellular matrix. It is not uncommon for these tumours to be diagnosed during gestation and their volumetric changes remain a matter of debate. The fibroid can be a source of obstetric complications in pregnant women and pregnancy can influence the condition of the fibroid. The objective is to evaluate the behaviour of fibroids coexisting with pregnancy according to their genetic profile in order to determine the penetrance of the *COMT* gene in Senegalese patients. We analysed the variability of the *COMT* gene in 6 pregnancy patients with fibroids by PCR-sequencing. Molecular analyses of the *COMT* gene show an involvement of exon 4 of the said gene in this pathology.

**Keywords:** fibroid; pregnancy; benign; *COMT*; Senegal

## Introduction

The terminology used to name the benign uterine pathology we are describing has not yet been given a single, definitive and consensual name, since the terms myoma, leiomyoma, fibroleiomyoma and, more frequently, fibroma are also used, which reflects the histopathological diversity of the condition [1]. Uterine fibroids are benign monoclonal neoplasms of the smooth muscle layer of the uterus [2,3]. Moreover, two essential features of leiomyomas are increased smooth muscle cell proliferation and excessive extracellular matrix deposition [4]. Common causes of fibroids are factors variables, such as genetics, endocrine factors and lifestyle factors [5,6]. It is now accepted that uterine fibroids are dependent on steroid hormones, including oestrogen [7]. However, myomatous uterine pathology is common, with approximately 25% of genitally active women being carriers. This means that there is a high probability of its interaction with pregnancy. The increase in the age of the first pregnancy, which increased from 24 years in 1978 to 30 in 2012, saw an increase in the occurrence of uterine fibroids during pregnancy [8,9]. Nevertheless, fibroids can have consequences probably on all stages of fetal development. In addition to affecting fertility by mechanically preventing conception and implantation, they can also complicate the course of pregnancy, delivery and postpartum. Pregnancy, on the other hand, can facilitate the progression of myomas to complications [10].

## Presentation of cases and methodology

The study was conducted in 6 pregnant Senegalese patients diagnosed with uterine fibroids. The age range was [28-41 years]. These patients are managed at the Maternity and Obstetrics Gynecology Departement of the General Hospital Idrissa Pouye after obtaining ethical approval (Reference:

Protocol 0267/2017/CER/UCAD). As fibroids are benign tumours, the surgeries were performed on the tumour tissue only. These samples were directly sent to the Genomics laboratory of the Population Genetics and Management Team at UCAD where they were stored in tubes containing 96% alcohol for molecular analysis. The gene related to the metabolism of the estrogen Catechol-O-Methyltransferase (*COMT*) encoded by a single gene in mammals, which in the human species is located in band q11.21 of chromosome 22 [11,12], was studied. The extraction of total DNA from each sample was done using the Zymo protocol (Zymo research kit). For electrophoretic migration, 5 µl of DNA extracts and 3 µl of bromophenol blue (loading blue), were deposited on a 2% agarose gel (with Safeview 10 µl) and migrated at 100 volts for 30 minutes. Exon 4 of the *COMT* gene is amplified from the DNA extracts made. Thus, PCR conditions were optimised for each primer pair (sense primer: 5'-TACTGTGGCTACTCAGCTGTGC-3' and the antisense primer 5'-GTGAACGTGGTGTGAACACC-3') and applied uniformly to all 6 samples in a 25 µl reaction volume. Amplification conditions were 94°C/3min; 35cycles at 93°C/45s, 55°C/1min, 72°C/4min; 72°C/4min and Hold at 10°C [12]. After sequencing, the result is presented in the form of a chromatogram representing the succession of bases making up the DNA fragment. The sequences obtained after sequencing were carefully checked, corrected and aligned with BioEdit version 8.0.5 software [13] to determine site homologies among others. The mutation search was done with the Mutation Surveyor version 5.0.1 software (www.softgenetics.com) which compares the submitted chromatograms with the reference sequence Accession (NG\_011526) of the said gene. To see the pathogenicity of substitutions in exon 4 of the *COMT* gene, the nucleotide sequences are

translated using MEGA7 [14] into protein sequences and these are submitted to SIFT (<http://www.sift.jcvi.org>), Mutation Taster (<http://www.mutationtaster.org>), Polyphen-2 (<http://www.genetics.bwh.harvard.edu/pph2>), and UMD Predictor (UMD ([umd-predictor.eu](http://umd-predictor.eu))). Genetic characterization is the determination of the population identity card in a global analysis that shows the number of sites, haplotypic and nucleotide diversity, mutation rate, percentage of transitions and transversions, total number of mutations, number of haplotypes, nucleotide frequencies as well as substitution types. The determination of these parameters was done with the Dnasp software version 5.10 [15]. However, the nucleotide frequencies, the nature of the mutations and the mutation rate are performed with the program MEGA version 7.0.14. The codon selection test, which allow us to see if the mutations are heterogeneously distributed, was performed with MEGA version 7.0.15 [14].

## Results

We analysed the variability of the *COMT* gene in 6 pregnancy patients. The alignment is done with the reference sequence LRG\_1010, Accession (NG\_011526), Version (NG\_011526.1).

## Mutation research

Chromatogram analysis shows the presence of mutations (c.408C>G, c.438C>T et c.472G>A) in the *COMT* gene (Figure 1) in 3 patients. The other 3 patients do not show any variability in the *COMT* gene.

All mutations found are listed in the dbSNP database.

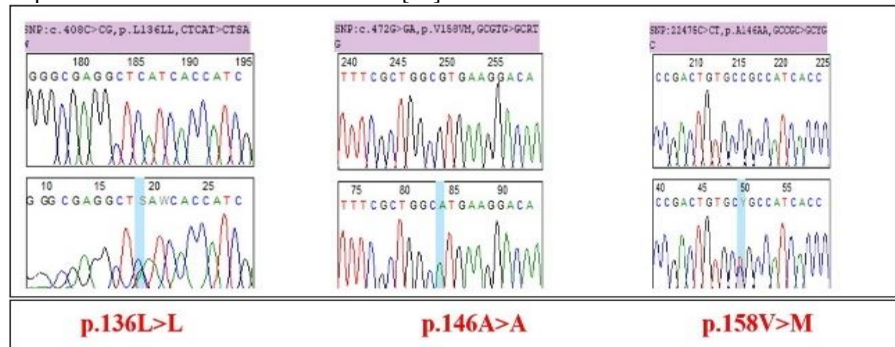


Figure 1: SNPs of exon 4 of the *COMT* gene in fibroids coexisting with pregnancy.

Most of the mutations in exon 4 of the *COMT* gene are synonymous mutations that do not induce an amino acid change and have no effect on coding, unlike the non-synonymous c.472G>A mutation that induces a substitution of valine for methionine (Table 1).

Fibroids and pregnancy			
Variants	dbSNP	Effect on coding	Amino acid assigned
c.408C>G	rs4818	Synonymous mutation	p.136L>L
c.438C>T	rs8192488	Synonymous mutation	p.146A>A
c.472G>A	rs4680	Missense mutation	p.158V>M

Table 1: Nature and position of the mutations.

## Pathogenicity of mutations

The non-synonymous variant c.472G>A is considered benign polymorphic by all software pathogenicity (Table 2).

Variants	UMD Predictor	SIFT (Score)	Mutation Taster (score)	Polyphen2 (score)
c.472G>A p.158V>M	Polymorph	Tolerated (0.12)	Missence (0.49)	Benign (0.005)

Table 2: Pathogenicity of mutations.

## Genetic characterisation

The amplified region of the *COMT* gene is 182pb. Three polymorphic sites were found, one of which is informative. The total number of mutations is 3. The number of haplotypes is 4. Concerning the diversity indices we note a

high haplotypic diversity (hd) and a low nucleotide differences k is 1.2. The mutation rate R is 2.621. The mutations in exon 4 of the *COMT* gene are much more transitional in nature (Table 3).

Parameters	Pregnancy patients with fibroma
Number of sequences	6
Number of sites	182
Monomorphic sites	179
Polymorphic sites	3
Singleton sites	2
Parcimony informative site	1
Total number of mutations (Eta)	3
Number of haplotypes	4
Haplotypic diversity (hd)	0.8 ± 0.02963
Nucleotide diversity (Pi)	0.0065 ± 0.0000040
Average number of nucleotide difference (k)	1.2
% transition	73.22
% transversion	26.8

Mutation rate (R)	2.621
A+T	39.83
C+G	60.17

Table 3: Parameters of diversity.

### Codon selection test

The codon selection test, which allows us to see if the mutations are heterogeneously distributed, shows that the difference between dN and dS

is zero for all codons except codons 1 and 31, where it is negative and codon 67, where it is greater than zero (Méthionine) with p-values >0,05. No codon is under selection, so the mutations are heterogeneously distributed (Table 4).

Codon	Triplet	dN-dS	p-value
1	CTC	-0.923	1
31	GCC	-1	1
67	ATG	0.399	0.83

Table 4: Codon selection test of exon 4 of the *COMT* gene.

### Discussion

In this report, the nuclear catechol-O-methyltransferase (*COMT*) gene involved in oestrogen metabolism was investigated in 6 cases of fibroids and pregnancy. The presence of mutations shows that the *COMT* gene is involved in the occurrence of uterine fibroids in Senegalese women. This is in agreement with the study by Cong *et al.* (2012) [16] which suggests that a large number of genes are associated with the risk of uterine leiomyoma, and the *COMT* gene is one of them. The c.472G>A (p.158V>M) polymorphism (rs 4680) (score 0.49) is found in 2 of the 3 patients with mutations. Thus the c.472G>A variant appears to be associated with the incidence of uterine fibroids in the Senegalese population. This differs from the study (PCR-pyrosequencing in genotyped women) by Denschlag *et al.* (2006) [17] which shows no statistically significant difference in allele frequency and distribution of the *COMT* G158A genotype in Caucasian women. *COMT* converts 2-hydroxyestradiol to 2-methoxyestradiol. 2-Hydroxyestradiol has been shown to act as an anti-estrogen in many tissue systems [18,19]. On the other hand, 2-methoxyestradiol has been shown to have a mitogenic effect on different cell types [20,21,22]. Therefore, the high activity *COMT* genotype (Val/Val) would result in a rapid and efficient conversion of the anti-estrogenic metabolite (2-hydroxyestradiol) into its more mitogenic counterpart (2-methoxyestradiol), creating a high estrogenic cellular environment. Conversely, the low activity *COMT* genotype (Met/Met) would lead to the accumulation of 2-hydroxyestradiol, creating a low estrogenic environment [23]. WHO has demonstrated lower levels of 2-hydroxyestradiol in leiomyomas compared to adjacent normal myometrium and implicated it in tumour genesis. According to Rutherford *et al.* (2008) and Bilder *et al.* (2004) [24,25] the Val158Met polymorphism (rs4680) is considered the main cause of variation in *COMT* activity. This single nucleotide (SNP), involving a G to A transition at codon 158, results in an amino acid change and makes the enzyme prone to active site distortion and protein aggregation at physiological temperature. We found 3 variable sites which stipulates that mutations in exon 4 of the said gene are not the only genetic alterations in uterine fibroids. The 4 haplotypes identified show that there is a similarity between individuals which could be explained either by tumour size or by the same tumour location. The percentage of transition is 26.8% so the *COMT* gene would be more likely to have mutations in individuals whose fibroids do not coexist with pregnancy. We note a high haplotypic diversity (hd) and a low nucleotide diversity (Pi) which shows a signal of population growth and therefore an increase in mutations over time. This could be explained by the fact that epidemiological factors such as age, obesity, parity, gestational age, etc. are important in the growth mechanism of fibroids. According to the codon selection test no codon is under selection. This shows that there are more synonymous mutations than non-synonymous mutations.

### Conclusion

The results of the molecular analyses obtained show an involvement of exon 4 of the *COMT* gene in uterine fibroids coexisting with pregnancy in Senegalese women. The Val158Met polymorphism (rs4680) is considered to be the main cause of *COMT* activity dysfunction.

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