Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/17134
DOI URL: http://dx.doi.org/10.21474/IJAR01/17134

RESEARCH ARTICLE

INVOLVEMENT OF THE *COMT* GENE IN THE OCCURRENCE OF UTERINE FIBROIDS INSENEGAL

Codou Diop, Bineta Keneme and Pape Mbacke Sembene
Laboratoire de Génomique, Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal.

Manuscript Info

Manuscript History
Received: 25 April 2023
Final Accepted: 28 May 2023
Published: June 2023

Key words:-
Fibroma, Mutation, *COMT*, Val158Met, Senegal

Abstract

Context. Uterine fibroids, also known as uterine leiomyomas, are monoclonal tumours of the smooth muscle tissu layer (myometrium). They are characterised by high levels of collagen and fibronectin and are most common in women of reproductive age. Despite the efforts made, the etiology fibroids remain unknow particulary in african women which present a higher risk than caucasian.
Objective.The objective is to evaluate the involvement of *COMT* gene mutations in the evolution of uterine fibroids in Senegalese women.
Methods. We analysed the variability of the *COMT* gene in 44 patients by PCR-sequencing.We proceeded first to the verification, cleaning, alignment of the sequences obtained, then to the search for mutations and their pathogenicity and finally to a genetic characterization with the aim of determining the penetrance of the *COMT* gene in the evolution of fibroids.
Results.Analysis show that the Val158Met polymorphism (rs4680) is considered to be the main cause of variation in *COMT*activity. Our data suggest that this polymorphism is a causal factor in the incidence of uterine fibroids by allowing a higher estrogenic activity in myometrial cells.

Introduction:-

Uterine fibroids, also known as myomas or leiomyomas, are benign monoclonal neoplasms of the smooth muscle cells of the uterus (Whiteman *etal*., 2010; Stewart *etal*., 2017).Two key features of leiomyomas are increased muscle smooth cell proliferation and excessive extracellular matrix deposition (Grudzien *etal*., 2010). Several factors could be involved in leiomyoma enlargement, including interactions between various genes, hormones, growth factors and cytokines although oestrogen and progesterone have traditionally been considered the main promoters of leiomyoma growth (Eude *etal*., 2001; Salama *etal*., 2009 and Grudzien *etal*., 2010).Tumour expansion is supported by cell proliferation as well as by the production of large amounts of extracellular matrix. Estrogen, with its Erα receptor, enables the action of progesterone via the induction of progesterone receptor (PR) expression. Progesterone induces leiomyoma growth through the regulation of a set of key genes that control proliferation and apoptosis(Bulun *et al*., 2015). However clinical diagnosis of fibroids can occur at all stages of disease development from small incidentally identified tumours to large symptomatic tumours (Divakar, 2008; Laughlin andStewart, 2011). Many are discovered incidentally on clinical (Cruz *etal*., 2017). Epidemiological data indicate that leiomyomas are virtually non-existent before menarche and generally have an indolent course after menopause, strongly implicating gonadal hormones in the induction and maintenance of this disease process (Lewis *et al*., 2018).Uterine fibroids do not affect all breeds

Corresponding Author:- Codou Diop
Address**:-** Populations Genetics, Departement of Animal Biology, Faculty of Science and Technology, Cheikh Anta Diop University, Dakar, Senegal.

equally. Essam *etal*., (2008) observed an increased prevalence of uterine fibroids in African American women. In a recent publication, Payson *etal*., (2008) developed an original vision of this tumour disease, the growth of which would correspond to a phenotype that would cover several genetic conditions, themselves related to several ethnic groups. Black race, heredity, obesity, diabetes and hypertension are associated with specific clinical presentations compared to families with no history of fibroids(Racinet, 2009).

Methods:-

Samples

The study is conducted on 44 Senegalese patients with uterine fibroids. Thesepatients are managed at the Maternity and Obstetrics Gynecology Departement of Idrissa Pouye General Hospital after obtaining ethical approval (Reference:Protocol 0267/2017/CER/UCAD). Uterine fibroids, being benign tumours therefore surgeries are performed on tumour tissu 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.

DNA Extraction, Polymerase Chain Reaction (PCR) and Sequencing

Total DNA from each sample is extracted using the Zymo protocol (Zymo research kit). The gene related to the metabolism of the estrogen Catechol-O-Méthyltransférase (*COMT*) encodes by a single gene in mammals, which in the human species is located in band q11.21 of chromosome 22 (Oliveira *et al*., 2008, Zhai *et al*., 2019) is studied. Exon 4 of the *COMT*gene is amplified. Thus, PCR conditions were optimised using primers 5'-TACTGTGGCTACTCAGCTGTGC-3' and 5'-GTGAACGTGGTGTGAACACC-3' in a reaction volume of 25 µl. The amplification conditions are: 94°C/3min; 35cycles at 93°C/45s, 55°C/1min, 72°C/4min; 72°C/4min and hold at 10°C (Oliveira *et al*., 2008).

The samples are sequenced in the ABI 3730xl sequencer (Appled Biosystems).

Mutation research

To determine the presence of any mutation and the position of the mutation in relation to the genes, the raw sequencing data for each of the genes is submittedto Mutation Surveyor version 5.0.1 (www.softgenetics.com) which compares the submitted chromatograms with the reference sequence Accession (NG_011526) of that gene.

Prediction of the pathogenesis of mutations

To see the pathogenicity of *COMT*exon 4 substitutions in these uterine fibroids, the nucleotide sequences are translated using MEGA7 software (Tamura *et al*., 2013) 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 UMDPredictor (UMD (umd-predictor.eu).

Comparison of mutation frequency

The*COMT*gene mutations found are compared to those found in other studies to see if there is any difference in gene expression between the study populations and the general population.

Genetic characterisation

Multiple alignment

The obtained sequences were thoroughly checked, corrected and aligned with BioEdit version 8.0.5 Hall, (1999) to determine site homologies among others. We proceeded first to the verification, cleaning, alignment of the obtained sequences, then to the search for mutations, their percentage and frequency and their pathogenicity and finally to a genetic characterisation with the aim of determining the penetrance of the *COMT* gene in the evolution of fibroids.

Analysis of genetic variability and genetic diversity

The genetic characterisation amounts to determining the identity card of the population in a global analysis that shows the number of sites, the sample size, the number of variable and invariant sites, the haplotypic and nucleotide diversity, the mutation rate, the percentage of transitions and transversions, the total number of mutations, the number of haplotypes, the nucleotide frequencies as well as the types of substitutions. The determination of these parameters was done with the DnaSP software version 5.10 (Librado and Rozas, 2009).However, the nucleotide frequencies, the nature of the mutations and the mutation rate are performed with the program MEGA version

7.0.14(Tamura *etal*., 2013). The codon selection test that allows us to see if the mutations are heterogeneously distributed was run with MEGA version 7.0.14(Tamura *etal*., 2013).

Results:-

We analysed the variability of the *COMT* gene in 44 patients with uterine fibroids by PCR-Sequencing. The alignment is done with the reference sequence LRG_1010, Accession (NG_011526), Version (NG_011526.1).

Mutation research

Chromatogram analysis showed the presence of mutations (c.408C>G, c.438C>T and c.472G>A) in the*COMT*gene(Figure 1) in 36 patients. The remaining 8 did not show any mutation.

All mutations found are listed in the dbSNP database.

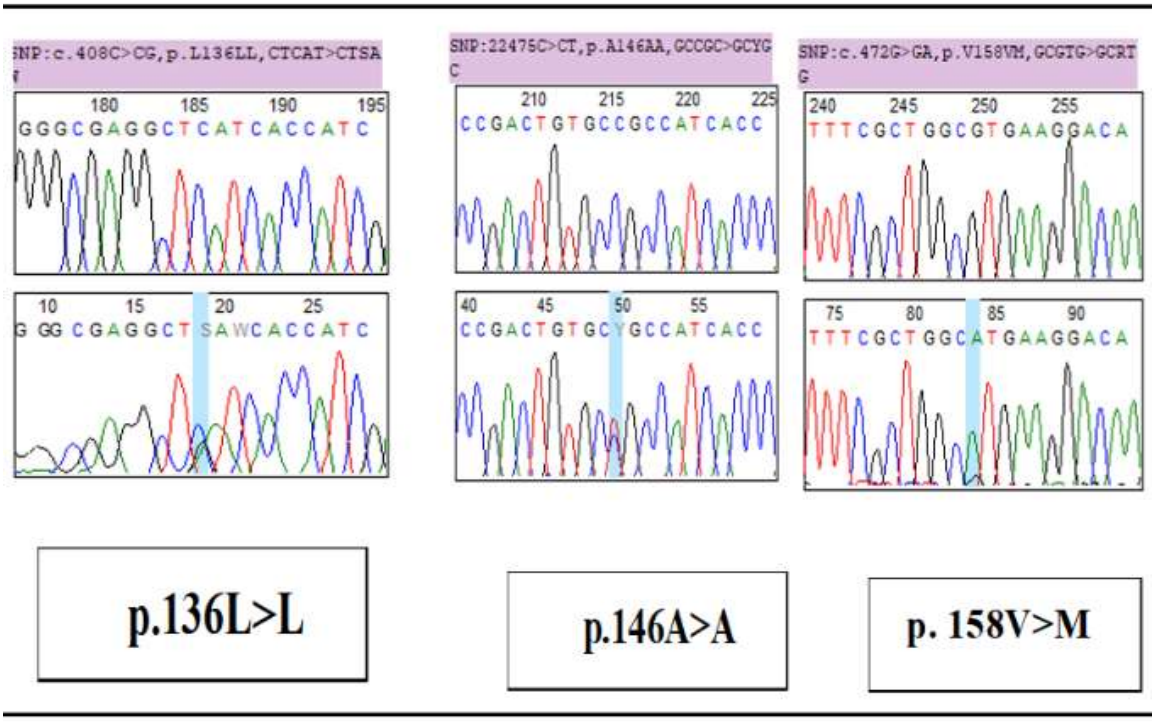

Figure 1:- SNPs in exon 4 of the *COMT*gene in uterine fibroids.

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).

Table 1:- Nature and position of mutations.

Uterine fibroid			
Variants	dbSNP	Nature	Amino acid
c.408C>G	rs4818	Synonymous mutation	p.136L>L
c.438C>T	rs8192488	Synonymous mutation	p.146A>A
c.472G>A	rs4680	Missence mutation	p.158V>M

Percentage of mutations

After alignment the gene region is 182 bp and shows 3 polymorphic sites (positions: 3 (c.408C>G), 33 (c.438C>T) and 67 (c.472G>A)). The classification of the 44 individuals according to the gene profile gives us the following results: 8 patients did not show any variability for exon 4 of the *COMT*gene,2patients showed 2 mutations at the same time, 6 patients have the c.408C >G (p.136L>L), 1 patient had the c.438C >T mutation (p.146A>A), and 26 had the c.472G >A mutation (p.158V>M) (Table 2).

Table2:- Percentage of mutations.

Variants	Number of patients	Percentage (%)
No presence of variants	8	18.18
c.408 C>G	6	13.63
c.438C>T	1	2.27
c.472G>A	26	59.09

Frequency of mutations

Compared to the general population (gnomad v2.1.1) the variant c.472G>A is more expressed in the population. The mutation frequency of Val158Met is higher in Senegalese than in European population and very low in European an South Asian population. This shows an differential expression of *COMT* polymorphism in population(Table3).

Table 3:- Frequency of mutations in exon 4 of the*COMT* gene.

Variants	c.408C>G	c.438C>T	c.472G>A
Afro/Afro-American	0.2003	0.085	0.3109
European (no-Finlandia)	0.3943	0.00067	0.5198
South Asian	0.3214	0.00013	0.4399
Senegal	0.159	0.0681	0.6136

Pathogenicity of mutations

The non-synonymous variant c.472G>Ais considered benign polymorphic by all software pathogenecity predictor (Table 4).

Table4:- Pathogenicity of mutations.

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)

Genetic characterisation

The amplified region of the*COMT*gene is 182 pb. Three polymorphic sites were found, three of which are informative. The total number of mutations is 3. The number of haplotypes is 6. Concerning the diversity indiceswe note a high haplotypic diversity (hd) and a low nucleotide diversity (Pi). The average number of nucleotide differences k is0.928. The mutation rate R is 2.413. Transition mutations are more frequent than transversion (Table5).

Table 5:- Parameters of diversity.

Parameters	Patients with uterine fibroids
Number of sequences	44
Number of sites	182
Monomorphic sites	179
Polymorphic sites	3
Singleton sites	0
Parcimony informative site	3
Total number of mutations (Eta)	3
Number of haplotypes	6
Haplotypic diversity (hd)	0.61 ± 0.00486
Nucleotide diversity (Pi)	0.0051 ± 0.0000005
Average number of nucleotide difference (k)	0.928
% transition	71.52
% transversion	28.48
Mutation rate (R)	2.413
A+T	39.95
C+G	60.05

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 they are negative and codon 67 where it is greater than zero (Valine) with p-value >0.05. No codon is under selection so the mutations are heterogeneously distributed (Table 6).

Table 6:- Codon selection test of exon 4 of the*COMT* gene.

Uterine fibroids			
Codon	Triplet	dN-dS	p-value
1	CTC	-1.817	1
31	GCT	-2	1
67	GTG	0.434	0.76

Discussion:-

In this study, the catechol-O-methyltransferase (*COMT*) gene involved in the metabolism of oestrogen was investigated in 44 Senegalese patients with uterine fibroids. The presence of mutations in these tissues shows that the *COMT*gene is involved in the occurrence of uterine fibroids in Senegalese women. According to Al-hendy and Salama, (2006) catechol-O-methyltransferase is a ubiquitous enzyme that catalysis the S-adenosyl-L-methionine dependant methyl conjugation of the hydroxyl groups of catechol estrogens. It is an essential enzyme in estrogen metabolism, therefore regulation of *COMT*activity may indirectly modulate the biological effects of estrogen and play an etiological role in leiomyoma formation. The c.472G>A (p.158V>M) polymorphism (rs 4680) missence variant (score 0.49) is found in more than the half of theSenegalese population and the other population also. Thus the c.472G>A variant appears to be associated with the incidence of uterine fibroids.

The exact relationship between the *COMT* gene polymorphism and leiomyoma is not yet clear. A common genetic polymorphism, the G to A transition at codon 158, resulting in a valine-methionine substitution, is associated with thermal instability and a fourfold decrease in enzyme activity (Mitrunen, 2001). *COMT* converts 2-hydroxyestradiol to 2-methoxyestradiol. 2-Hydroxyestradiol has been shown to act as an anti-estrogen in many tissue systems(Bradlow, 1996 and Vandewalle, 1989). On the other hand, 2-methoxyestradiol has been shown to have a mitogenic effect on different cell types(Liu and Zhu, 2004; Banerjee et al., 2003; Lippert *et al*., 2003). Therefore, the high activity *COMT* genotype (Val/Val) would infer a rapid and efficient conversion of the anti-estrogenic metabolite (2-hydroxyestradiol) to its more mitogenic counterpart (2-methoxyestradiol), thus creating a high estrogenic cell environment. Conversely, the low activity *COMT* genotype (Met/Met) would lead to the accumulation of 2-hydroxyestradiol, creating a low estrogenic environment. As uterine leiomyomas are oestrogen dependent, a higher frequency of occurrence would be associated with the Val/Val *COMT* genotype than the low oestrogen Met/Met genotype. However, the high expression of this polymorphism in the population, confirming the involvement of the *COMT* gene in fibroids in Senegalese women via oestrogen receptors, since fibroids are oestrogen dependent. The differences observed in relation to this Val158Met polymorphism could be explained by the difference in the group studied. Recent research has revealed that *COMT*expression and function are influenced by various factors such as genetic polymorphism, gender, age and diet. Notably, human *COMT*is highly polymorphic and more than 100 *COMT*alleles are thought to regulate its expression and catalytic activity (Carneiro *et al*., 2016 and Duursen *et al*., 2004). The most common*COMT*polymorphisms are Val158Met of MB-*COMT*andVal108Met of S-*COMT*, which can be used to predict enzyme activities (Lachman, 2008 and Sak 2017).Regarding the genetic characterisation, 3 variable sites were found, which stipulates that mutations in exon 4 of the said gene are not the only genetic alterations in cases of uterine fibroids and therefore other mutations would be involved. Out of 44 samples 6 haplotypes were identified which could be explained by a high similarity between individuals due to either the same size or location of the tumour. There was a high haplotype diversity (hd) and a low nucleotide diversity (Pi) which indicates a signal of population growth, thus an increase in mutations over time. This could be explained by the fact that epidemiological factors such as age, obesity, parity and gestational age play an important role in the growth mechanisms of fibroids. According to (Ciebiera *et al*., 2018 and Mohammadi *et al*., 2020), the most important and frequently reported risk factor for uterine fibroids is race, which disproportionately affects African American women. Other risk factors include advanced age, premenopausal status, non-parity, family history of uterine fibroids, hypertension, food additives, and frequent consumption of soy milk. Other important risk factors include obesity (23-25),vitaminD deficiency, excessive levels of vitamin E(Ciebiera *et al*., 2018), altered reproductive microbiome (Baker *et al*., 2018),exposure to endocrine disruptors (Lee *et al*., 2020 and Bariani *et al*., 2020), and various harmful environmental exposure in early life (Yang *et al*., 2018).The associations between uterine

leiomyoma and the Val158Met polymorphism of the *COMT* gene have been studied in different ethnic populations with conflicting results. Genotype frequencies of the *COMT*polymorphism in different populations have shown variations due to ethnicity (Ivanova *et al*., 2010; Morikawa *et al*., 2008; Gooden *et al*., 2007). The ethnic disparity in the incidence and biological behaviours of uterine leiomyoma suggests that different races may have differences in estrogen biosynthesis and/or metabolism (Othman and Al-Hendy, 2008). The difference in allele distribution between the Senegalese and other populations could be related to patients' fibroid types, histological variants or risk factors such as parity or gestational age. Most of the patients in this study are nulligravida and nulliparous, as the fibroid is oestrogen-dependent and nulliparous women who have not breastfed have hyperoestrogenism which could induce the occurrence of fibroid.

Conclusion:-

In conclusion, our data suggest that exon 4 of the *COMT*gene is involved in the occurrence of uterine fibroids in Senegalese women and the c.472G>A (p.158V>M) polymorphism (rs4680) represents a causal factor in the incidenceof uterine fibroids by allowing a higher estrogenic activity in myometrial cells.

Bibliographic References:-

1.**Al-Hendy A, Salama SA.**Catechol-O-methyltransferase polymorphism is associated with increased risk of uterine leiomyoma in different ethnic groups. Journal of the Society for Gynecologic Investigation. 2006;13(2):136-144. Doi:10.1016/j. jsgi.2005.10.007.

2. **Baker, J. M., Chase, D. M., & Herbst-Kralovetz, M. M**. (2018). Uterine microbiota: residents, tourists or invaders? Frontiers in immunology, 9, 208.

3. B**anerjee SN, Sengupta K, Banerjee S, Saxena NK, Banerjee SK.** 2-Methoxyestradiol exhibits a biphasic effect on VEGF-A in tumor cells and upregulation is mediated through ER-alpha: a possible signaling pathway associated with the impact of 2-ME2 on proliferative cells. Neoplasia. 2003; 5 :417–426.

4. **Bariani, M. V., Rangaswamy, R., Siblini, H., Yang, Q., Al-Hendy, A., & Zota, A. R.** (2020). The role of endocrine disrupting chemicals in the pathogenesis of uterine fibroids. Current opinion in endocrinology, diabetes, and obesity, 27(6), 380.

5. **Bradlow LH**. 2-Hydroxyesterone: the "good" estrogen. J Endocrinol. 1996; 150: S259–S265.

6. **Bulun, S. E., Moravek, M. B., Yin, P., Ono, M., Dyson, M. T., Navarro, A., ... & Wei, J. J.** (2015, September). Uterine leiomyoma stem cells: linking progesterone to growth. In Seminars in reproductive medicine (vol. 33, no. 05, pp. 357-365). Thieme Medical Publishers.

7. **L.S. Carneiro, A.M. Fonseca, P. Serrao, et al.,** Impact of physical exercise on catechol-O-methyltransferase activity in depressive patients: a preliminary communication, J. Affect. Disord. 193 (2016) 117e122.

8. **Ciebiera, M., Szymańska-Majchrzak, J., Sentkowska, A., Kilian, K., Rogulski, Z., Nowicka, G., ... & Włodarczyk, M.** (2018). Serum alpha-tocopherol levels are increased in Caucasian women with uterine fibroids: a pilot study. BioMed Research International, 2018.

9.**Ciebiera, M., Włodarczyk, M., Ciebiera, M., Zaręba, K., Łukaszuk, K., & Jakiel, G.** (2018). Vitamin D and uterine fibroids - a review of the literature and emerging concepts. International Journal of Molecular Sciences, 19(7), 2051.

10. **De La Cruz, M. S. D., et Buchanan, E. M.** (2017). Uterine fibroids: diagnostic et treatment. Am. Fam. physician 95 (2), 100-107.

11. **Divakar, H.** (2008). Asymptomatic uterine fibroids. Best Pract. Res. Clin. Obstetrics Gynaecol. 22 (4), 643-654. Doi: 10.1016/j.bpobgyn.2008.01.007.

12. **van Duursen, M. B., Sanderson, J. T., de Jong, P. C., Kraaij, M., & van den Berg, M.** (2004). Phytochemicals inhibit catechol-O-methyltransferase activity in cytosolic fractions of healthy human breast tissue: implications for catechol estrogen-induced DNA damage. Toxicological Sciences, 81(2), 316-324.

13. **Eude, I., Dallot, E., Vacher-Lavenu, M. C., Chapron, C., Ferre, F., & Breuiller-Fouche, M**. (2001). Potentiation response of cultured human uterine leiomyoma cells to various growth factors by endothelin-1: role of protein kinase C. European journal of endocrinology, 144 (5), 543-548.

14. **Othman, E. E. and Al-Hendy, A.** (2008). Molecular genetics and racial disparities in uterine leiomyomas. Best practices and research. Clinical obstetrics & gynaecology, 22(4), 589-601. https://doi.org/10.1016/j.bpobgyn.2008.01.014.

15.**Gooden KM, Schroeder JC, North KE, Gammon MD, Hartmann KE, Taylor J, Baird DD** (2007) Val153Met polymorphism of catechol-o-methyltransferase and prevalence of uterine leiomyomata. Reprod Sci 14:117–120.

16.**Grudzien, M. M., Low, P. S., Manning, P. C., Arredondo, M., Belton Jr**. The antifibrotic drug halofuginone inhibits proliferation and collagen production by human leiomyoma and myometrial smooth muscle cells. Fertility and Sterility, 93(4), 1290-1298.
17. **Hall TA** (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.Nucl. Acids. Symp. Ser. 41 :95-98.
18.**H.M. Lachman**, Does COMT val158met affect behavioral phenotypes: yes, no, maybe?Neuropsychopharmacology 33 (2008) 3027e3029.
19.**Ivanova TI, Kondrashova TV, Krikunova LI, Smirnova IA, Shentereva NI, Sychenkova NI, Rykova EV, Zharikova IA, Khorokhorina VA, Riabchenko NI, Zamulaeva IA** (2010) The polymorphism of catechol-o-methyltransferase (COMT) and hemochromatosis (HFE) genes in the radiocontaminated regions residents with different chromosome aberration frequency. Radiats Biol Radioecol 50:117–127.
20. **Laughlin, S. K., & Stewart, E. A.** (2011). Uterine leiomyomas: individualizing the approach to a heterogeneous condition. Obstetrics & Gynecology, 117(2 Part 1), 396-403.
21. **Lee, G., Kim, S., Bastiaensen, M., Malarvannan, G., Poma, G., Casero, N. C., ... & Choi, K.** (2020). Exposure to organophosphate esters, phthalates and alternative plasticizers in association with uterine fibroids. Environmental Research, 189, 109874.
22. **Lewis, T. D., Malik, M., Britten, J., San Pablo, A. M., & Catherino, W. H.** (2018). A comprehensive review of the pharmacological management of uterine leiomyoma. BioMed research international, 2018, 2414609. https://doi.org/10.1155/2018/2414609.
23. **Librado, P. and Rozas, J.** (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11), 1451-1452.
24. Liu Z-J, Zhu BT. Concentration-dependent mitogenic and antiproliferative actions of 2-methoxyestradiol in estrogen receptor positive human breast cancer cells. J Steroid Biochem Mol Biol. 2004; 88:265–275.
25. Lippert C, Seeger H, Mueck AO. The effect of endogenous estradiol metabolites on the proliferation of human breast cancer cells. Life Sci. 2003; 72:877–883.
26. Mitrunen K. Polymorphic catechol-O-methyltransferase gene and breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2001; 10:635–640.
27. **Mohammadi, R., Tabrizi, R., Hessami, K., Ashari, H., Nowrouzi-Sohrabi, P., Hosseini-Bensenjan, M., & Asadi, N.** (2020). Correlation between low serum vitamin D and uterine leiomyoma: systematic review and meta-analysis. Reproductive Biology and Endocrinology, 18, 1-8.
28.**Morikawa J, Yoshida S, Kennedy S, Takemura N, Sugimoto M, Kitao K, Deguchi M, Ohara N, Maruo T** (2008) Association study between catechol-o-methyltransferase polymorphisms and uterine leiomyomas in a Japanese population. Clin Exp Obstet Gynecol 35:35–40.
29. **de Oliveira, E., de Aquino Castro, R., Gomes, M. T. V., da Silva, I. D. C. G., Baracat, E. C., de Lima, G. R., ... & Girão, M. J. B. C.** (2008). The catechol-O-methyltransferase (*COMT*) gene polymorphism and prevalence of uterine fibroids. Maturitas, 60(3-4), 235-238.
30.**Othman EE, Al-Hendy A** (2008) Molecular genetics and racial disparities of uterine leiomyomas. Best Pract Res Clin Obstet Gynaecol 22:589–601.
31. **Payson, M., Leppert, P. et Segars, J.** (2006). Epidemiologicalof myoma. Obstetrics and gynecology clinics of North America, 33(1), 1-11. https://doi.org/10.1016/j.ogc.2005.12.004.
32. **Racinet, C.** (2009). Epidemiology, risk factors and symptomatology of uterine myomas. Medecine de la Reproduction, 11(2), 118-122.
33**. K. Sak**, The Val158Met polymorphism in *COMT* gene and cancer risk: role of endogenous and exogenous catechols, Drug Metab. Rev. 49 (2017) 56e83.
34.**Salama, S. A., Kamel, M. W., Botting, S., Salih, S. M., Borahay, M. A., Hamed, A. A., ... & Diaz-Arrastia, C.** R. (2009). Catechol-o-methyltransferase and 2-methoxyestradiol expression affects microtubule dynamics and alters steroid receptor signaling in leiomyomatous cells. PLoS One, 4(10), e7356.
35. **Stewart, E. A., Cookson, C. L., Gandolfo, R. A., & Schulze-Rath, R.** (2017). Epidemiology of uterine fibroids: a systematic review. BJOG: An International Journal of Obstetrics & Gynaecology, 124(10), 1501-1512.
36. **Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S.** (2013). MEGA6: molecular evolutionary genetic analysis version 6.0. Molecular biology and evolution, 30(12), 2725-2729.
37.Vandewalle B. Opposite effects of estrogen and catecholestrogen on hormone-sensitive breast cancer cell growth and differentiation. Mol Cell Endocrinol. 1989; 61:239–246.
38. **Whiteman, M. K., Kuklina, E., Jamieson, D. J., Hillis, S. D. and Marchbanks, P. A**. (2010). Hospitalization for gynecologic disorders in the United States. American journal of obstetrics and gynecology, 202(6), 541-e1.

39.**Yang, Q., Ali, M., El Andaloussi, A., & Al-Hendy, A**. (2018). The emerging spectrum of early exposure-related inflammation and epigenetic therapy. Cancer studies and molecular medicine: open journal, 4(1),13.
40. **Zhai, J., Jiang, L., Wen, A., Jia, J., Zhu, L., & Fan, B**. (2019). Analysis of the relationship between *COMT* polymorphisms and endometriosis susceptibility. Medicine, 98(1).