Probing Genetics and Environmental Factors underlying Uterine Fibroid Tumorigenesis in Ghana, West Africa

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Academic editor: Editorial Secretary

Abstract

Uterine fibroid (UF) is the most prevalent benign tumour that affects millions of women globally, with a high incidence of 70% amongst women of reproductive age. UF has been associated with various complications, such as recurrent surgeries, infertility, anemia and pregnancy loss. Notably, women of African descent often experience more severe symptoms and complications. Although hormones, growth factors, and genetic alterations are widely associated with UF, the precise mechanism underlying its pathogenesis is not fully understood. Recent evidence suggests altered microbiota may serve as a potential risk factor for UF development. Altered microbiota can contribute to tumorigenesis via epigenetic changes to host cells or toxic effects from invasion. The lack of curative-drug treatment poses significant challenges to patients with UF. Patients often undergo surgeries that require the removal of the uterus or tumour, which can negatively impact fertility. Furthermore, uterine fibroids' diagnosis relies on expensive imaging technologies such as ultrasound, which may not be readily available in developing countries. Moreso, diagnosis is often conducted only after patients' symptoms become severe. Although late presentation may contribute to severe symptoms and complications among women with UF in Africa, other factors that influence severity and increase incidence in this population remain unknown. A comprehensive assessment of UF predisposing factors in high-risk populations such as Ghana could give better insights into disease pathogenesis. Hence, this study aims to assess: UF-associated demographic factors, the role of uterine microbiota dysbiosis on UF tumorigenesis; and molecular markers associated with UF in the Ghanaian population. Epidemiological data and clinical samples (tissues, blood and cervico-vaginal swabs) will be obtained. The characterization of samples will involve metagenomics, whole genome sequencing, functional validation of SNPs and SNP genotyping. The association of risk alleles with disease phenotypes will be assessed via regression analysis using PLINK v.1.9. The

findings will provide information on potential disease markers that can be explored for better management strategies for UF in high-risk populations.

Keywords

uterine fibroids, reproductive tract infections, metagenomics, altered microbiota

Introduction

Uterine fibroids (UFs) are non-malignant pelvic tumours of the uterine smooth muscle that affect 70% of women in their pre-menopausal years (Igboeli et al. 2019). Globally, UF results in infertility, recurrent pregnancy loss, heavy menstrual bleeding, dyspareunia and frequent urination (Navarro et al. 2021). Approximately 65% of women with UF are asymptomatic and late presentation of the condition sometimes results in early pregnancy failure and complications such as placenta abruption and preterm labour (Chill et al. 2019). Despite the associated clinical outcomes, long-term non-invasive treatment options are not available for fibroids resolution. The condition therefore generates a massive healthcare burden in terms of surgeries, mostly hysterectomy (complete surgical removal of the uterus) and myomectomy (removal of tumors) and prolonged hospital stays (Stewart et al. 2016). Recurrent fibroids that require recurrent myomectomy are seen in 20% of UF cases (Kuznetsova et al. 2022). UFs are the cause of over 40 – 60% of all gynecological surgeries in African countries like Ghana, Cameroon and Nigeria (Opare-Addo et al. 2014, Egbe and Egbe 2018). Additionally, Ghana reports fibroids as one of the top 10 causes of hospitalisation over the past five years (Ofori-Dankwa et al. 2019).

UFs affect a wide cross-section of the population; however, African women show a higher prevalence, early onset and rapidly growing number of tumors compared to other races (Al-Hendy et al. 2017). Genetic predispositions have been implicated in UF, especially in developed countries. Genetic variation, such as single nucleotide polymorphisms that may be associated with fibroids susceptibility, has been identified (Cha et al. 2011). However, the pathogenesis of UF is not understood due to the heterogeneity of fibroids. There is also a paucity of information on the genetic basis of the increased incidence of fibroids; previous studies focused more on Caucasians and a few African Americans but none on native Africans (Eltoukhi et al. 2014). African Americans' genome is approximately 80% African due to admixture (Bryc et al. 2015). Therefore, identified UF variants in such populations may be attributable to admixture and may have no contributing effects on the increased risk of UF in the African population.

Studies have identified environmental risk factors for UF in an effort to understand its etiology. In Ghana, studies using retrospective data identified age, obesity, parity and history of reproductive tract infections (RTI) as putative risk factors for UF (Opare-Addo et al. 2014). There is a need for a more comprehensive study to gain insights into the prevalent environmental factors that predispose Ghanaian women to UF. Recent

evidence in developed countries also suggests altered microbiota as a risk factor (Mao et al. 2022). Such alterations from the direct effect of microbes or the cross-talk of their metabolites can alter the immune system, host gene expression and metabolic signalling (Sobstyl et al. 2022). There is a high burden of RTI in developing countries (Torrone et al. 2018). In addition, heterologous bacteria can translocate from one organ to colonise other tissues or organs and influence human health (Wang et al. 2021). Considering the aforementioned, there is a need to investigate the uterine microbiota as a risk factor for UF in this population.

Overall, the genetic and other host-related basis of UF pathogenesis remain underexplored in high-risk populations such as Africa. Therefore, a comprehensive assessment of factors that predispose Ghanaian women to UF development is critical. A population-based study integrating environmental and genetic factors will provide knowledge on how these factors influence genetic predisposition and UF development. The outcome of this study will provide information for candidate biomarkers for UF prevention, diagnosis and treatment and ultimately improve management strategies.

Research Questions

This study will answer the following questions:

1. What are the non-genetic risk factors predisposing Ghanaian women to UF?
2. How does the alteration of the uterine microbiota contribute to UF tumorigenesis?
3. What are the unique genetic variants in the Ghanaian population associated with UF?

Study aims and Objectives

Aim 1: To investigate the influence of epidemiological factors and the uterine microbial community on fibroid tumorigenesis amongst Ghanaian women:

- Identify the association of demographic factors and clinical manifestations of UF amongst Ghanaian women;
- Determine the uterine microbial profile associated with UF;
- Analyse the role of patient-derived microbiome on fibroid growth *in-vitro*.

Aim 2: To profile molecular markers associated with fibroid tumorigenesis:

- Characterise and identify variants associated with fibroid tumorigenesis;
- Functionally analyse identified SNPs *in-vivo*;
- Validate the unique disease markers in extended cohort.

Background and Study Impact

BACKGROUND

Uterine Fibroids (UF)

Uterine fibroids, also called leiomyoma, are benign tumour growths that arise from the proliferation and differentiation of single cells. UF growth can be influenced by genetic and non-genetic factors (Fig. 1). Histologically, UF-associated markers are smooth muscle fibres with increased extracellular matrix (ECM) contents, mainly fibronectin, proteoglycans and collagen (Mas et al. 2014). Fibroids can grow as single or multiple tumours in the uterine wall (myometrium), with sizes ranging from pea to watermelon. They occupy different layers of the uterus and are subtyped as intramural, submucosal and subserosal. Depending on the location and lesion size, symptoms range from pelvic pain, heavy bleeding, anaemia and frequent urination to miscarriages, preterm birth and infertility (Bulun 2013). In Africa, the majority of women appear with huge fibroids and seek medical attention late due to inadequate access to proper healthcare services and high costs (Igboeli et al. 2019). In Ghana, there is a paucity of information on the burden of UF; nonetheless, a significantly high level of UF cases has been reported in a tertiary hospital (Ofori-Dankwa et al. 2019). Age, obesity and a history of sexually transmitted infections have been associated with UF development in the country (Opare-Addo et al. 2014).

Aetiology and Risk factors

Characteristic risk factors associated with UF pathogenesis include African descent, family history and early menarche (Fig. 2). In spite of differences in racial and ethnic background, age is consistently linked to the incidence of UF. Women between 40 and 60 years old are at a 4–11 times higher risk of developing UF than women between 20 and 30 and those above the age of 60 (Morhason-Bello and Adebamowo 2022). An increase in oestrogen and progesterone regulating genes hormone markers also results in the proliferation of UF cells (Lora et al. 2012). A low level of vitamin D is also an UF risk factor and the black race has a higher risk of deficiency (Ciebiera et al. 2021).

Obesity is an established risk factor for UF and it is characterised by the secretion of adipokines and chronic inflammation. Inflammatory cytokines could lead to increased levels of reactive oxygen species, promote the proliferation of UF cells and enhance ECM deposition.

Altered Microbiome and Epigenetic regulation

Microbiome (microbiota and its environment) is known to play a role in human health and disease. The microbiota in the reproductive tract of women is important in the regulation of reproduction, a major physiological process in mammals (Heil et al. 2019). The uterine layer has always been considered sterile; however, sequencing technology revealed it is colonised by a unique microbiota that, when altered, is associated with various disease states (Peric et al. 2019). Recently, bacterial communities have been seen to ascend to the uterine cavity from the vagina through the cervix, resulting in microbiome imbalance (

Wang et al. 2021). Such colonisation, as evidenced by the co-occurrence of multiple organisms, the outgrowth of beneficial species and the release of virulence factors, may alter host gene expression and induce epigenetic change. These changes in the UF genome activate signalling pathways that play an essential role in UF pathologic processes (Mas et al. 2014). There is no study on the uterine microbiota and its role in UF development. Shotgun metagenomics can give insights on how microbial communities influence disease processes by interacting with the host.

Genetic Alterations of UF

Genetic factors are major players in UF development. Findings via genome-wide linkage analysis and SNP arrays in non-African populations identified a proportion of genetic variants associated with UF (Cha et al. 2011). The most closely associated variant with a high risk of UF development is mutations in the mediator complex subunit 12 (MED12). Mutations in this gene arise under the influence of infection or mechanotransduction and result in dysfunction of the metabolic pathway associated with ECM formation and myoblast proliferation (Baranov et al. 2019). Others include rearrangement of the high mobility group AT-hook gene (HMGA1), collagen type IV alpha 5 and 6 (COL4A5 and COL4A6) deletion and biallelic inactivation of fumarate hydratase – FH - that result in autosomal dominant syndrome (Baranov et al. 2019). Some variants in non-Africans were not recurrent in African-Americans, indicating fibroid heterogeneity and involvement of different pathways in UF tumorigenesis from different regions (Mehine et al. 2016). High-throughput sequencing technology provides nearly full coverage of variations in the genome and can contribute to unknown aetiology. There are little or no genetic studies on UF pathogenesis in Africa. Hence, a population-based study to identify and experimentally validate population-specific UF-associated variants would be an optimal approach. The poor pathophysiology of UF has resulted in limited identification of drug targets that can prevent UF development in high-risk groups.

Therapeutics and Diagnostics

Currently, UF treatment options and preventative strategies are limited. Available invasive surgical procedures require lengthy recovery time, they are associated with complications and are not suitable for women who desire fertility. The non-invasive options lessen UF-associated symptoms; however, they present side effects and require a perpetual regimen. Additionally, there is a risk of tumour size increase or fibroid regrowth following cessation of treatment (Aninye and Laitner 2021). The rate of UF recurrence following myomectomy after the initial removal of all existing fibroids is high. Prevention strategies, such as early diagnosis and prophylaxis treatment for women at high risk, are necessary.

Diagnosis of UF is done by imaging procedures (pelvic and transvaginal ultrasound, MRI) that are expensive and not readily available in low-resource areas. However, cell free DNA (CfDNA) detected in body fluids (plasma and serum) has recently emerged as a diagnostic and prognostic marker for benign and malignant disorders (Ganesamoorthy et

al. 2022). Available serum biomarkers that can accurately diagnose UF will improve early-stage diagnosis, aid routine screening, prevent misdiagnosis and may predict women's risk of recurrent UF.

Study Impact

Uterine fibroids result in high morbidity in Africa and oftentimes mortality from treatment-related complications, especially blood loss during myomectomy (Igboeli et al. 2019). Frequent exposure and translocation of pathogens due to factors such as RTI could alter the uterine-wall microbiota and initiate pathways that can provoke the development of tumour. Previous studies on leiomyoma etiology focused on hormones and genetic predisposition, but none considered the potential role of the altered uterine microbiota. The mechanism behind the increase fibroids incidence in the African population remains understudied. There are little or no biological studies on UF genetic predispositions in the African population. To date, only hormonal treatment exists as a non-invasive treatment option due to the limited knowledge of disease mechanisms. An integrated approach that considers trait-associated genetic variants and environmental risk factors can provide information for biomarkers for prophylaxis, diagnostic tools and precise treatment. This will increase UF management efficiency and reduce morbidity and the associated socio-economic burden. Overall, this study will contribute to the understanding of the etiology of UF tumorigenesis in a high-risk population. Consequently, we will overcome the public health problem.

Implementation

Study workflow

To achieve the study objectives, the following workflow (Fig. 3) will be adopted.

Study Design

This will be a case-control study using clinically-confirmed UF patients and non-affected individuals from selected regions in Ghana (Fig. 4) based on cluster sampling (Xiong et al. 2004). Epidemiological mapping will be generated online and on-site.

Ethical Clearance

Study approval will be obtained from all sample sites and Ghana Health Service Ethics Review Committee. The study participants will be counselled and signed informed consent will be obtained before enrolment into the study. Ethical approval will also be obtained from the Institutional Animal Care and Use Committee, Noguchi for animal studies.

Participation will be voluntary and study participants will be anonymised using unique study identification codes and all information treated with strict confidentiality.

Sample size determination

The sample size will be determined using the Cochran formula, where d is precision level, Z is critical value and P is sample proportion. In this study, a proportion of 70% will be used because probability is 70% in pre-menopausal women.

Sample calculation will be done as shown in:

$n = Z^2P(1-P)/d^2$

$n = (1.96)^2(0.70)(1-0.70)/0.05^2$

$n = 322.4 \sim 350$

Therefore, the sampling distribution will be as shown (Fig. 5)

Sample Collection

Women aged 20 - 60 years with or without UF in the selected sites enrolled into the study will be provided with a questionnaire. Those within the reproductive age with confirmed UFs scheduled for hysterectomy or myomectomy in selected hospitals will form the cases, while those admitted for hysterectomy for any other indications apart from UF will form the control. Those who have not attained menarche and unwilling to participate will be excluded. Fibroid tissue and normal adjacent myometrium tissue, blood and cervico-vaginal swabs will be obtained from cases, while blood and cervico-vaginal swabs obtained from controls (Fig. 5). Tumour number and sizes will be recorded and vitamin D level measured in the serum.

Assessment of demographic factors and clinical manifestations of UF in Ghanaian women

This objective will assess age, parity, body mass index (BMI), family history and UF-related basic clinical features from enrolled participants using the survey questionnaire (Suppl. material 1). Data will be grouped and associations with UF will be determined accordingly.

Determining uterine microbial profile from UF cases and controls

This objective will assess differences in microbial type and abundance. Samples will be grouped into those with or without RTI.

Sample preparation

Swab and tumour tissue homogenate (PDM) will be suspended in 2 ml of phosphate buffer solution (PBS) and centrifuged (5 min, 12000 rpm). Nucleic acids will be extracted from pellets using the microbiome DNA/RNA extraction kit.

Shotgun metagenomics sequencing and data analysis

Metagenomics sequencing will be carried out as described previously (Quince et al. 2017). Briefly, libraries will be prepared using Nextera XT library kit then sequenced using NextSeq platform (Illumina). Bacteria taxonomic classification will be conducted from sequenced data after a quality check using Kraken2 and paraKraken for differential viral particles**.** Data will be analysed using the workflow below (Fig. 6). Differences in taxa abundance will be considered statistically significant if adjusted p-values are < 0.05.

Role of patient-derived microbiome on fibroid tumorigenesis

The effect of microbiome on uterine fibroid cells (UtLm) and primary uterine smooth muscle cells (HUtSMC) will be measured after infecting the cells with PDM.

Cell Culture

This will be carried out as previously described (Rabiei et al. 2019). Cells will be cultured in Dulbecco Modified Eagle's Medium (DMEM) under the following conditions: 37°C, 5% CO_2, and 95% humidity. At 80% confluence, cells will be detached using trypsin and then centrifuged. Pellets will be reconstituted in DMEM and 100 ul will be transferred into a 24-well plate at a density of $5x10^4$ cells per well. Patient-derived microbiome (PDM) will be added to the cells using the various cell-culture conditions A-E (Fig. 7) and then incubated and monitored for cell viability**.**

Cell Viability assay

Viability media solution will be added after discarding the culture medium for viability determination. After 4 h incubation, the culture media will be discarded and solubilisation solution will be added into each well and optical density of viable cells will be measured (550-630 nm) using a microplate reader.

Immunohistochemical analysis

UF-associated markers including alpha smooth muscle actin (ACTA2), desmin, COL1 and COL4 (COL1-A, COL-94) will be measured. Proliferation, angiogenesis, apoptotic and hormone markers will be measured using primary antibody (anti-Ki67, anti-VEGF, anti-Bcl-2, anti-ER, anti-PR), respectively. Briefly, cells will be blocked in 3% bovine serum albumin for 1 h, followed by overnight incubation in the primary antibody. Secondary antibody conjugated to substrate will be added for enzymatic detection.

Profiling molecular markers associated with UF

This objective aims to identify UF-associated genetic variants amongst Ghanaian women using group 1 clinical samples (Fig. 5).

Sample Preparation

DNA from tissue samples will be extracted using AllPrep Universal kit, while total RNA will be extracted using Qiazol reagent following the manufacturer's instructions. Blood samples (5 ml) will be centrifuged (10 min, 3000 rpm) to separate blood cells and plasma. Plasma aliquots will be re-centrifuged (10 min, 13000 rpm) and used for Plasma cfDNA extraction using the Circulating Cell free Nucleic acid kit.

Genomics profiling of UF tissue and controls using whole exome sequencing

Whole exome sequencing will be carried out as described in McGuire et al. (2012). DNA samples from tissue and blood will be subjected to in-solution exome enrichment using SureSelect Human All Exon Kit. Samples will be sent for sequencing (Illumina platform) following exome capture. Data will be aligned to the human genome GRCh37, quality control and downstream variant analysis will be performed. Focus will be on variants present in the fibroids, but absent in adjacent normal myometrium.

Variant calling and annotation

Variant filtering and quality control will be carried out using NextGENe software. Somatic single nucleotide variants (SNV) will be detected from sequenced data using VarScan2. Already-existing SNV associated with UF in literature will be searched for and used as control. Copy number aberrations (CNA) will be detected using IchorCNA and CNA from matched germline samples will serve as control. Structural variant (SV) calling will be observed using Manta.

Molecular annotation

SIFT and polyphen2 will be used to identify SNPs that will have an impact on protein function. These non-synonymous SNPs will further be validated *in-vivo*. To identify disease-causing genes commonly mutated in fibroids, variants will be compared across samples. These genes will be analysed for their biological functions using DAVID and ReactomePA to identify pathways impacted due to functional alteration of the genes.

RT-qPCR

The relative expression of identified disease-causing genes and existing UF driver genes in literature will be measured using RT-qPCR from normal and diseased tissues. The mRNA levels will be normalised using the endogenous gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The relative fold change in gene expression will be computed using the $2^{-\Delta\Delta Ct}$ formula.

Functional analysis of identified marker *in-vivo*

This objective sought to identify if the non-synonymous SNPs are disease-causing mutations using experimental xenograft model and CRISPR-Cas9 technology**.**

Design of ssDNA/sgRNA/Cas9 expression vectors

Single-stranded donor oligonucleotides (ssDNA) carrying desired mutations will be generated via site-directed mutagenesis and used as DNA repair templates. Cas9 mRNA, single guide RNAs (sgRNAs) with 20 bp of homology flanking either sides of the target integration sites and ssDNA will be synthesised from Biogene. The promoter driving expression of gene's sgRNA will also be cloned into the backbone plasmid as previously described (Luo et al. 2018). Mutation will be confirmed using PCR and sequencing. The plasmids will be co-transfected in HutSMc and UtLm. Scrambled vector and non-transfected cells will serve as control.

Xenograft transplantation

To mimic human UF microenvironment, animals bearing UF xenograft will be used as previously described (Wang et al. 2021). Briefly, 4-5 weeks old germ-free female SCID mice will be purchased and grouped into two (six each), test and control group. The mice will be anaesthetised using chloroform inhalation. Cells (HutSMc, UtLm and mutants (ΔUtLm, ΔHutSMc)) mixed with matrigel will be grafted beneath the kidney capsule following subcutaneous implantation of oestradiol. They will be monitored daily; developed lesions and body weight will be measured twice per week using an electronic scale and vernier caliper.

Morphological and histological evaluation

Mice will be euthanised after 8 weeks by CO_2 inhalation and tumours will be dissected and evaluated.

Genotyping the unique marker associated with UF

This will be done to further validate SNPs in the remaining subset of clinical samples, Group 2 (Fig. 5) and also confirm if SNPs are in circulation for blood-based diagnosis.

SNP genotyping

SNPs that show a strong association with the UF disease phenotype will be genotyped in CfDNA and tissue DNA. Specific primers will be designed for each polymorphism and samples will be amplified using the restriction fragment length polymorphism (PCR) approach and targeted genotyping.

Bioinformatics and Statistical Analyses

Bioinformatics analysis will be performed using packages anchored in R (version 4.1.3) as detailed in the Methods section. For statistical analysis, data will be subjected to normality test using the Shapiro-Wilk test. Association between assessed variables and UF will be determined using the Chi-square test. Measurements between two groups or amongst more than two groups will be compared using the t-test and ANOVA, respectively. Disease marker association will be determined using PLINKv.1.9. All tests will be two-tailed, significance level will be set at 5% and p-value less than 0.05 will be interpreted as significant. The crude and adjusted odds ratio and CI of 95% will be noted.

Expected outcomes

The epidemiological study will identify the prevalence of UF and provide knowledge on how factors (age, obesity, vitamin D levels, parity and family history) contribute to an increased risk of UF amongst Ghanaian women. The microbiome study will give insights into microbial groups that are risk alleles for UF and the role of the uterine microbiota in modulating the physical health of women with regards to fibroid development. This can influence the use of host gene expression and microbial profile as active surveillance biomarkers.

The genetic studies will identify unique UF-associated genetic variants in this population. In addition, essential genes and relevant molecular pathways that are activated or dysregulated in fibroid tissue compared to normal tissue will be identified. These can be harnessed for the design of preventative and fertility-friendly treatments for women of reproductive age. A functional study will provide insights on the function of SNPs and their availability in blood. Cell free DNA biomarker SNP array chips can be developed for UF diagnosis as well as predicting the risk of developing UF.

Acknowledgements

The authors would like to acknowledge the Head of the Department of Biochemistry, Cell and Molecular Biology, Professor Osbourne Quaye, the staff of the Department, especially Dr. Lucas Amenga-Etego and Professor Lydia Mosi. The PhD fellows: Alexandra Lindsey Djomkam, Charles Olwal Ocheng, Godwin Nsoh Anabire and Kesego Tapela for their support and West African Centre for Cell Biology of Infectious Pathogens for the opportunity.

Hosting institution

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Conflicts of interest

The authors have declared that no competing interests exist.

References

- Al-Hendy A, Myers ER, Stewart E (2017) Uterine Fibroids: Burden and Unmet Medical Need. Seminars in Reproductive Medicine 35 (6): 473-480. https://doi.org/10.1055/s-0037-1607264
- Aninye I, Laitner M (2021) Uterine Fibroids: Assessing Unmet Needs from Bench to Bedside. Journal of Women's Health (2002) 30 (8): 1060-1067. https://doi.org/10.1089/jwh.2021.0280
- Baranov V, Osinovskaya N, Yarmolinskaya M (2019) Pathogenomics of Uterine Fibroids Development. International Journal of Molecular Sciences 20 (24). https://doi.org/10.3390/ijms20246151
- Bryc K, Durand E, Macpherson J , Reich D, Mountain J (2015) The Genetic Ancestry of African Americans, Latinos, and European Americans across the United States. American Journal of Human Genetics 96 (1): 37-53. https://doi.org/10.1016/j.ajhg.2014.11.010
- Bulun S (2013) Uterine fibroids. The New England Journal of Medicine 369 (14): 1344-1355. https://doi.org/10.1056/NEJMra1209993
- Cha P, Takahashi A, Hosono N, Low S, Kamatani N, Kubo M, Nakamura Y (2011) A genome-wide association study identifies three loci associated with susceptibility to uterine fibroids. Nature Genetics 43 (5): 447-450. https://doi.org/10.1038/ng.805
- Chill HH, Karavani G, Rachmani T, Dior U, Tadmor O, Shushan A (2019) Growth pattern of uterine leiomyoma along pregnancy. BMC Women's Health 19 (1). https://doi.org/10.1186/s12905-019-0803-5
- Ciebiera M, Ali M, Prince L, Zgliczyński S, Jakiel G, Al-Hendy A (2021) The Significance of Measuring Vitamin D Serum Levels in Women with Uterine Fibroids. Reproductive Sciences 28 (8): 2098-2109. https://doi.org/10.1007/s43032-020-00363-8
- Egbe TO, Egbe TO (2018) Prevalence and Outcome of Hysterectomy at the Douala General Hospital, Cameroon: A Cross-Sectional Study. Int J Surg Res Pract https://doi.org/10.23937/2378-3397/1410092
- Eltoukhi H, Modi M, Weston M, Armstrong A, Stewart E (2014) The health disparities of uterine fibroid tumors for African American women: a public health issue. American Journal of Obstetrics and Gynecology 210 (3): 194-199. https://doi.org/10.1016/j.ajog.2013.08.008
- Feng Y, Ramnarine VR, Bell R, Volik S, Davicioni E, Hayes V, Ren S, Collins C (2019) Metagenomic and metatranscriptomic analysis of human prostate microbiota from patients with prostate cancer. BMC Genomics 20 (1). https://doi.org/10.1186/s12864-019-5457-z
- Ganesamoorthy D, Robertson AJ, Chen W, Hall M, Cao MD, Ferguson K, Lakhani S, Nones K, Simpson P, Coin LM (2022) Whole genome deep sequencing analysis of cell-free DNA in samples with low tumour content. BMC Cancer 22 (1). https://doi.org/10.1186/s12885-021-09160-1

- Heil B, Paccamonti D, Sones J (2019) Role for the mammalian female reproductive tract microbiome in pregnancy outcomes. Physiological Genomics 51 (8): 390-399. https://doi.org/10.1152/physiolgenomics.00045.2019
- Igboeli P, Walker W, McHugh A, Sultan A, Al-Hendy A (2019) Burden of Uterine Fibroids: An African Perspective, A Call for Action and Opportunity for Intervention. Current opinion in gynecology and obstetrics 2 (1): 287-294. https://doi.org/10.18314/cogo.v2i1.1701
- Kuznetsova M, Sogoyan N, Donnikov A, Trofimov D, Adamyan L, Mishina N, Shubina J, Zelensky D, Sukhikh G (2022) Familial Predisposition to Leiomyomata: Searching for Protective Genetic Factors. Biomedicines 10 (2). https://doi.org/10.3390/biomedicines10020508
- Lora V, Grings AO, Capp E, von Eye Corleta H, Brum IS (2012) Gene and protein expression of progesterone receptor isoforms A and B, p53 and p21 in myometrium and uterine leiomyoma. Archives of Gynecology and Obstetrics 286 (1): 119-124. https://doi.org/10.1007/s00404-012-2245-2
- Luo X, Deng C, Su X, Wang F, Chen Z, Wu X, Liang S, Liu J, Fu L (2018) Loss of MED12 Induces Tumor Dormancy in Human Epithelial Ovarian Cancer via Downregulation of EGFR. Cancer Research 78 (13): 3532-3543. https://doi.org/10.1158/0008-5472.CAN-18-0134
- Mao X, Peng X, Pan Q, Zhao X, Yu Z, Xu D (2022) Uterine Fibroid Patients Reveal Alterations in the Gut Microbiome. Frontiers in Cellular and Infection Microbiology 12 URL: https://www.frontiersin.org/articles/10.3389/fcimb.2022.863594
- Mas A, Cervello I, Gil-Sanchis C, Simón C (2014) Current understanding of somatic stem cells in leiomyoma formation. Fertility and Sterility 102 (3): 613-620. https://doi.org/10.1016/j.fertnstert.2014.04.051
- McGuire M, Yatsenko A, Hoffner L, Jones M, Surti U, Rajkovic A (2012) Whole Exome Sequencing in a Random Sample of North American Women with Leiomyomas Identifies MED12 Mutations in Majority of Uterine Leiomyomas. PloS one 7 https://doi.org/10.1371/journal.pone.0033251
- Mehine M, Kaasinen E, Heinonen H, Mäkinen N, Kämpjärvi K, Sarvilinna N, Aavikko M, Vähärautio A, Pasanen A, Bützow R, Heikinheimo O, Sjöberg J, Pitkänen E, Vahteristo P, Aaltonen L (2016) Integrated data analysis reveals uterine leiomyoma subtypes with distinct driver pathways and biomarkers. Proceedings of the National Academy of Sciences of the United States of America 113 (5): 1315-1320. https://doi.org/10.1073/pnas.1518752113
- Morhason-Bello I, Adebamowo C (2022) Epidemiology of uterine fibroid in black African women: a systematic scoping review. BMJ Open 12 (8). https://doi.org/10.1136/bmjopen-2021-052053
- Navarro A, Bariani MV, Yang Q, Al-Hendy A (2021) Understanding the Impact of Uterine Fibroids on Human Endometrium Function. Frontiers in Cell and Developmental Biology 9 URL: https://www.frontiersin.org/article/10.3389/fcell.2021.633180
- Ofori-Dankwa Z, Ibine B, Ganyaglo GK (2019) The Uterine Fibroid Disease Burden in a Tertiary Care Setting in Ghana: Prevalence, Cost, and Policy Implications [7C]. Obstetrics & Gynecology 133 https://doi.org/10.1097/01.AOG.0000559428.84633.12
- Opare-Addo HS, Owiredu WKBA, Dapilah T, Alhassan A (2014) Putative Risk Factors among Ghanaian Women Presenting with Leiomyoma. Journal of Advances in Medicine and Medical Research5678-5688. https://doi.org/10.9734/BJMMR/2014/11823

- Peric A, Weiss J, Vulliemoz N, Baud D, Stojanov M (2019) Bacterial Colonization of the Female Upper Genital Tract. International Journal of Molecular Sciences 20 (14). https://doi.org/10.3390/ijms20143405
- Quince C, Walker A, Simpson J, Loman N, Segata N (2017) Shotgun metagenomics, from sampling to analysis. Nature Biotechnology 35 (9): 833-844. https://doi.org/10.1038/nbt.3935
- Rabiei P, Mohabatkar H, Behbahani M (2019) Studying the effects of several heat-inactivated bacteria on colon and breast cancer cells. Molecular Biology Research Communications 8 (2): 91-98. https://doi.org/10.22099/mbrc.2019.33958.1413
- Sobstyl M, Brecht P, Sobstyl A, Mertowska P, Grywalska E (2022) The Role of Microbiota in the Immunopathogenesis of Endometrial Cancer. International Journal of Molecular Sciences 23 (10). https://doi.org/10.3390/ijms23105756
- Stewart E, Laughlin-Tommaso S, Catherino W, Lalitkumar S, Gupta D, Vollenhoven B (2016) Uterine fibroids. Nature Reviews. Disease Primers 2 https://doi.org/10.1038/nrdp.2016.43
- Torrone E, Morrison C, Chen P, Kwok C, Francis S, Hayes R, Looker K, McCormack S, McGrath N, van de Wijgert JHM, Watson-Jones D, Low N, Gottlieb S, STIMA Working Group (2018) Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. PLoS medicine 15 (2). https://doi.org/10.1371/journal.pmed.1002511
- Wang J, Li Z, Ma X, Du L, Jia Z, Cui X, Yu L, Yang J, Xiao L, Zhang B, Fan H, Zhao F (2021) Translocation of Vaginal Microbiota Is Involved in Impairment and Protection of Uterine Health. Nature Communications 12 (1). https://doi.org/10.1038/s41467-021-24516-8
- Xiong LS, Chen MH, Chen HX, Xu AG, Wang WA, Hu PJ (2004) A population-based epidemiologic study of irritable bowel syndrome in South China: stratified randomized study by cluster sampling. Alimentary Pharmacology & Therapeutics 19 (11): 1217-1224. https://doi.org/10.1111/j.1365-2036.2004.01939.x

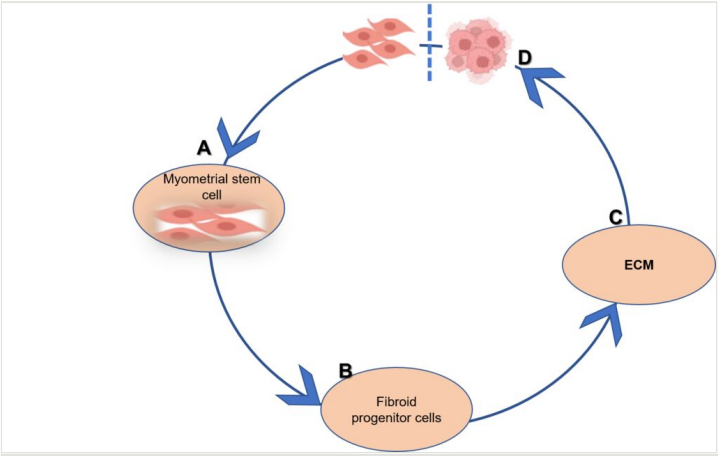

Figure 1.

Illustration of UF disease formation, **A** is transformed into a progenitor cell when influenced by environmental and genetic factors. **B** differentiates into four primary cell types: fibroblast, fibroid-associated fibroblast, smooth muscle cells and vascular smooth muscle cells which then produce **C** extracellular matrix (ECM) of the fibroids. Further influence of environmental and molecular factors promote the growth and proliferation of these cells and consequently, the clinically relevant fibroids (**D**).

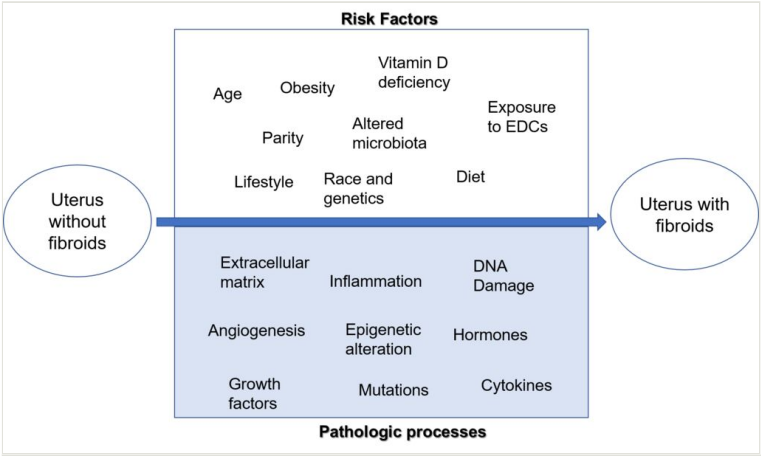

Figure 2.

Illustration of uterine fibroid risk factors and consequent pathologic processes, figure designed by author.

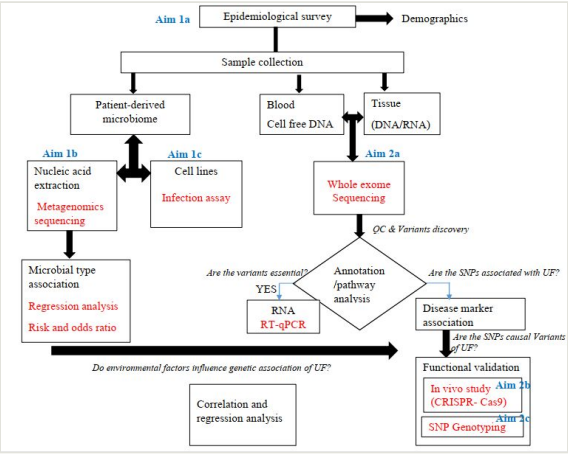

Figure 3.

Schematic representation of the study workflow.

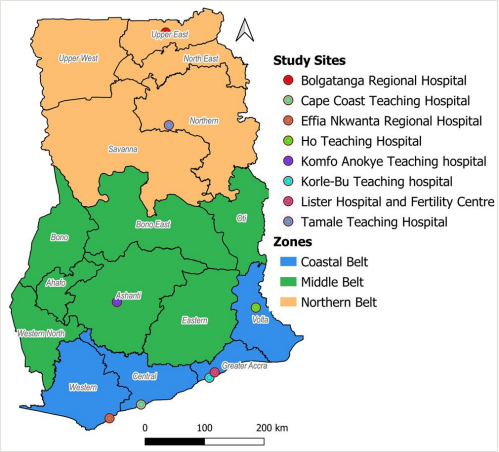

Figure 4.

Sample sites and hospitals selected from different clusters.

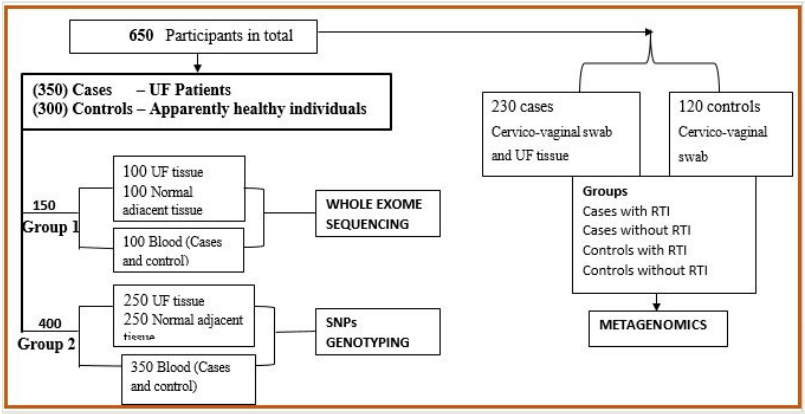

Figure 5.

Sampling Distribution. Abbreviations: UF means uterine fibroids, RTI means reproductive tract Infections.

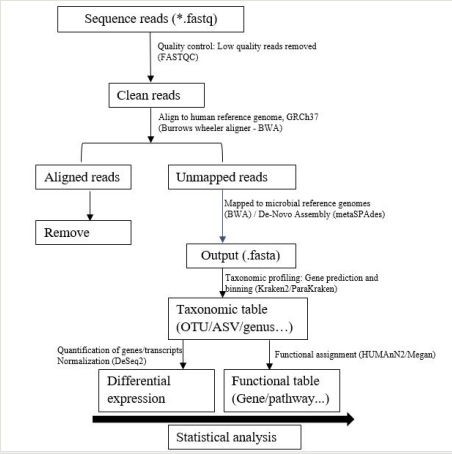

Figure 6.

Metagenomics data analysis workflow (adapted from Feng et al. (2019)).

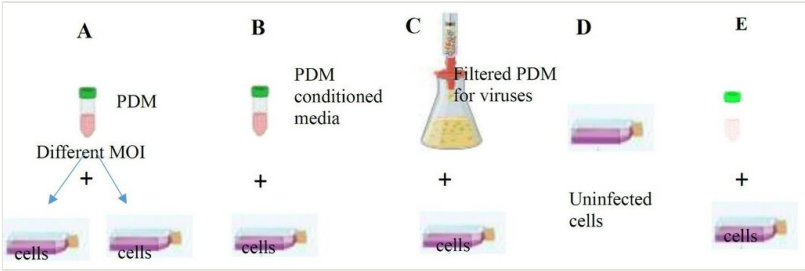

Figure 7.

Patient-derived microbiome (PDM) cell-culture conditions.

Supplementary material

Suppl. material 1: Survey questionnaire

Authors: Senbadejo, Tosin
Data type: Word document
Brief description: Survey questionnaire that covers important covariates including age, parity, family history, basic clinical features, common complaints and symptoms related to occurrence of fibroids. It will be administered via online and onsite methods.
Download file (27.34 kb)