WEST AFRICAN CENTRE FOR CELL BIOLOGY OF INFECTIOUS PATHOGENS
DEPARTMENT OF BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY
COLLEGE OF BASIC AND APPLIED SCIENCES
UNIVERSITY OF GHANA

GENETIC STUDIES OF SPONTANEOUS PRETERM BIRTH IN GHANAIAN WOMEN

BY:

PETER NURO-AMEYAW

(10329510)

OCTOBER 2024

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GENETIC STUDIS OF SPONTANEOUS PRETERM BIRTH IN GHANAIAN WOMEN

A dissertation submitted to the Board of Graduate Studies, University of Ghana, Legon, Ghana

In partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Biochemistry

BY

PETER NURO-AMEYAW

(10329510)

October 2024

DECLARATION

I, Peter Nuro-Ameyaw, of the department of biochemistry, Cell, and Molecular Biology, hereby declare that with the exception of the cited references, all information provided in this study was carried out the Department of biochemistry, Cell, and Molecular Biology. The study was conducted under the supervision of Dr. Lily Paemka (University of Ghana), Dr. Lucas Amenga-Etego (University of Ghana) and Prof. Kwame Adu-Bonsaffoh (University of Ghana).

25-10-2024

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Peter Nuro-Ameyaw Date

(Candidate)

25-10-2024

…….. …….

Dr. Lily Paemka Date

(Supervisor)

26-10-2024

…….. ……..

Dr. Lucas Amenga-Etego Date

(Supervisor)

28th October 2024

…….. ……..

Prof. Kwame Adu-Bonsaffoh Date

(Co-Supervisor)

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DEDICATION

This study is dedicated to all preterm children who have died as a result of inadequate research output and health infrastructure.

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11-HSD2	11β-hydroxysteroid dehydrogenase type 2
ADCY5	*ADENYLYL CYCLASE TYPE 5* gene
ADHD	Attention Deficit Hyperactive Disorder
AGTR2	*ANGIOTENSIN II RECEPTOR TYPE 2* gene
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal care
APC	Adenomatous Polyposis Coli
AUROC	Area Under the Receiver Operating Characteristic Curve
aOR	adjusted odds ratio
bp	base pair
β-TrCP	Beta-Transducin Repeat Containing E3 Ubiquitin Protein Ligase
CAPs	contraction-associated proteins
CAMKII	calcium-modulated kinases
CD	Cluster of differentiation
CES-D	Centre for Epidemiological Studies-Depression
cGMP	cyclic guanosine monophosphate
CK1	Casein Kinase 1
COL4A1	*COLLAGEN, TYPE 4, ALPHA 1*
COL4A2	*COLLAGEN, TYPE 4, ALPHA 2*
COL4A3	*COLLAGEN, TYPE 4, ALPHA 3*
COX	*CYCLOOXEGENASE GENE*
CRH	corticotropin releasing hormone
CX	connexin

DAAM	Dishevelled-Associated Activator of Morphogenesis
DAG	Diacylglycerol
DAMPs	Damage-associated molecular patterns
DHA	Docosahexaenoic acid
DMR	Differentially methylated region
DNA	Deoxyribonucleic Acid
Dvl	Dishevelled
EBF1	*EARLY B-CELL FACTOR 1* gene
ECM	extracellular matrix
EEFSEC	*EUKARYOTIC ELONGATION FACTOR, SELENOCYSTEINE-TRNA SPECIFIC* gene
EOC	epithelial ovarian cancer
EPA	Eicosapentanoic acid
ER	endoplasmic reticulum
Ets	E26 transformation-specific
fms	*Feline Mcdonough Sarcoma*
Fe^{2+}	ferrous ion
$Fe^{3+.}$	Ferric ion
FLT1	*FELINE MCDONOUGH SARCOMA-RELATED TYROSINE KINASE*
FSH	follicle-stimulating hormone
GDHS	Ghana Demographic and Health Survey
GETFund	Ghana Education Trust Fund
GHS	Ghana Health Service
GR	glucocorticoid receptor
GnRH	gonadotropin releasing hormone

GRCh38	Genome Reference Consortium human genome build 38
GRE	glucocorticoid response elements
GSK3β	Glycogen Synthase Kinase 3
GSS	Ghana Statistical Services
GWAS	genome-wide association studies
H&E	Haematoxylin and Eosin
HIV	Human Immunodeficiency Virus
H_2O_2	hydrogen peroxide
H19	*H19 Imprinted Maternally Expressed Transcript* gene
HC	Head circumference
hCG	human chorionic gonadotropin
HPA	hypothalamus-pituitary-adrenal
HIC	High income countries
HIF-1a	hypoxia-inducible factor-1a
hPL	human placental lactogen
HSE	heat shock element
HSF1	heat shock factor 1
HSP47	HEAT SHOCK PROTEIN 47
HSP47	*HEAT SHOCK PROTEIN 47* gene
HSPA1L	*HEAT SHOCK PROTEIN FAMILY A MEMBER 1-LIKE* gene
IDT	Integrated DNA Technologies
IGF2	*INSULIN-LIKE GROWTH FACTOR 2* gene
IL	*INTERLEUKIN* gene
IL	INTERLEUKIN protein

IP3	Inositol trisphosphate
IPV	Intimate partner violence
IUGR	intrauterine growth retardation
JNK	c-Jun N-terminal Kinase
KO	knock-out
LEF	lymphoid enhancer-binding factor
LGA	large for gestational age
LH	luteinizing hormone
LIFR-AS1	*LEUKAEMIA INHIBITORY FACTOR RECEPTOR ANTISENSE RNA 1*
LMIC	Lower-to middle-income countries
LRP	Low-Density Lipoprotein Receptor-related Protein receptors
MAPK	Mitogen-Activated Protein Kinase
MEG3	*MATERNALLY EXPRESSED GENE 3* gene
MEST	*MESODERM-SPECIFIC TRANSCRIPT HOMOLOG PROTEIN* gene
MMP	*MATRIX METALLOPROTEINASE* gene
MMP	MATRIX METALLOPROTEINASE protein
mRNA	messenger Ribonucleic acid
NCBI	National Center for Biotechnology Information
NFAT	Nuclear Factor of Activated T
NF-κB	Nuclear Factor Kappa B
NNAT	*NEURONATIN* gene
•OH	hydroxyl radicals
OR	Odds Ratio
P4H	*PYROLYL 4-HYDROXYLASE*

PAVM	placental accelerated villous maturation
PCP	Planar Cell Polarity
PCR	polymerase chain reaction
PDI	protein disulphide isomerase
PEG3	*paternally expressed gene 3*
PEG10	*paternally expressed gene 10*
PGE2	prostaglandin E2
PGF2α	prostaglandin F2α
$PGH_{2.}$	prostaglandin H_2
PKC	Protein Kinase C
PLAGL1	*pleiomorphic adenoma gene-like 1*
PLC	Phospholipase C
PMA	phorbol 12-myristate 13-acetate
pmol	picomolar
PPROM	preterm premature rupture of membrane
Rac1	Ras-Related C3 Botulinum Toxin Substrate 1
RFLP	restriction fragment length polymorphism
ROCK	Rho-Associated Coiled-Coil Kinase
PRKCA	*PROTEIN KINASE C—α* gene (human)
RAP2C	*RAT SARCOMA (RAS)-RELATED PROTEIN 2C* gene
RNA	Ribonucleic acid
rpm	revolutions per minute
SBP2	Selenocysteine Insertion Sequence binding protein 2
Sec	selenocysteine

SECIS	Selenocysteine Insertion Sequence
SERPINHI	*SERPIN FAMILY H MEMBER 1* gene
SGA	small for gestational age
SGCE	*ε-sarcoglycan gene*
SNP	Single nucleotide polymorphism
SPTB	spontaneous preterm birth
SPTL	Spontaneous preterm labour with intact membrane
TCF	transcription factors T-cell factor
TLE	Transducin-Like Enhancer of Split
TIMP	*TISSUE INHIBITORS OF METALLOPROTEINASE* gene (human)
TIMP	TISSUE INHIBITORS OF METALLOPROTEINASE protein (human)
UCSC	University of California, Santa Cruz
UK	United Kingdom
Unicef	United Nations International Children's Emergency Fund
VUE	Villitis of unknown aetiology
WACCBIP	West Africa Centre for Cell Biology of Infectious Pathogens
WNT	*Wingless-type Mouse Mammary Tumour Virus integration site family member*
WHO	World Health Organisation
WT	wild-type

Abstract

Background: Live birth before 37 gestation weeks, also known as preterm birth affects approximately 13.4 million births globally. Complications of preterm birth are responsible for about 1 million child deaths worldwide and lead to neonatal death every hour in Ghana. Approximately, two-thirds of preterm births occur spontaneously. However, the aetiology of spontaneous preterm birth (SPTB) is poorly understood. African women have the highest incidence of SPTB globally, even if environmental and socioeconomic conditions are adjusted for, suggesting a peculiar genetic predisposition. Genetic studies of SPTB in Africa are virtually unavailable. Understanding the genetic linkage will enhance the development of appropriate interventions. The study sought to identify specific phenotypes and genetic variants associated with SPTB among Ghanaian women. It was based on the hypothesis that SPTB among Ghanaian women is associated with a unique genetic variant.

Methods: The use of human subject for this study was approved by Ghana Health Service Ethics Review Committee with additional permission from all study centres. Informed consent was documented for all study participants prior to recruitment. The participants were recruited from seven health facilities across Ghana. Participants with acute infection and/or non-communicable diseases were excluded. A case-control study design was used involving 604 post-partum mothers with singleton births. Cases were 277 mothers with SPTB and controls were 327 mothers with term birth. Gestational ages of 24 to 42 weeks were considered. The demographic and phenotypic characteristics were identified through health records and structured interview questionnaire. Maternal psychological distress was assessed using the CES-D Scale. Maternal knowledge of their personal or immediate family (sister and mother) history of preterm birth was also assessed via interviews. Neonatal anthropometries including birth weight, head

circumference, length, and first and fifth minute Apgar scores were assessed. Placental samples were also collected for histological and RT-qPCR assessment. Whole blood (5 ml) was collected from each mother for DNA isolation and analysis. Six polymorphisms *SERPINH1* (- 656 C/T), *ADCY5* rs9861425 (A/C), *AGTR2* rs5950491 (C/A), *MMP-1* (-1607 1G/2G), *EEFSEC* rs2955117 (G/A) and *WNT-4* were genotyped using restriction fragment length polymorphism (RFLP). Univariate and multivariate analysis were conducted using the open-source statistical software R.

Result: Maternal age less than 20 years, less than antenatal 4 visits and depression were independently associated with SPTB by about 2-fold. Nulliparity, moderate Hb (Hb 9.9 – 7.0 g/dl) and "Not married status" were associated with increased SPTB risk by 64%, 60% and 71%, respectively but the associations were not independent. Chorioamnionitis was detected in 12.2% of placental samples. Its prevalence was high among SPTB placentas (16%) compared with 8.82% among term birth placentas. However, the difference was not statistically significant ($p < 0.07$). Indicators of maternal placental malperfusion were also detected in 6 out of 10 SPTB placental tissues. Ghanaian neonatal anthropometries (birth weight, head circumference and length) started dipping and diverged from that of the Olsen curve from the 29th gestation week till delivery either as SPTB or term. The first minute Apgar score of 0 – 3 was 7.1% and reduced to 3.5% in the fifth minute. The 7 – 10 Apgar scores increased from 67.4% in the first minute to 79.3% in the fifth minute.

The association between four genetic markers (*AGTR2* rs5950491, *WNT4* rs56318008, *EEFSEC* rs2955117 and *ADCY5* rs9861425) identified in the European genome-wide association studies to be associated with gestational duration was not replicated in this study. The *WNT4* rs56318008 (C/T) was found to be monomorphic with the CC genotype among Ghanaian

women. The T allele frequency of the *SERPINH1*- 656 (C/T) SNP was 0.1 in among Ghanaian women. The T allele of the *SERPINH1*- 656 (C/T) SNP and associated with over 2 days decrease in gestational duration, and 63% and 90% increased risk of SPTB and PPROM respectively in Ghanaian women. The *SERPINH1*- 656 (C/T) SNP was also associated with 67% and 77% increased risk of SPTB based on the heterozygous and additive models respectively. The TT genotype frequency was 0.0038 in the Ghanaian women population. It was observed only among women with SPTB, specifically those with PPROM. The TT genotype of the *SERPINH1*- 656 (C/T) SNP reduced the expression on *SERPINH1* in the placenta at a fold change of 0.77 compared with 0.94 for the CT (cases) as well as 1.07, 1.20 and 1.05 for the CT (controls), CC (control) and CC (cases) respectively. However, the reduced TT expression was not significantly different from the other genotypes ($p = 0.12$). Generally, the *MMP1* (-1607 1G/2G) SNP was not associated with SPTB except the heterozygous 1G/2G genotype that provided a 34% suggestive risk to SPTB but did not reach statistical significance ($p < 0.08$). Two out of 3 Ghanaian women with SPTB do not know whether they were born preterm or term. Over 7 out of 10 Ghanaian women with SPTB do not know whether either their mother and/or sister(s) was born preterm or term.

Conclusion: SPTB among Ghanaian women is influenced by both genetic and environmental factors. SPTB is independently influenced by maternal factors like age, antenatal visits and psychological distress. Signs of reduced rate of intrauterine development of Ghanaian foetuses is evident in the 29th week of gestation. The T allele of *SERPINH1*- 656 (C/T) could be a genetic risk associated gestational duration with PPROM occurrence in Ghana. A multi-omic study of SPTB will provide a very comprehensive understanding of the aetiology of SPTB in Ghana. Additionally, a comprehensive transgenerational study embedded with lifestyle characteristics is

needed to better understand how SPTB runs through families in Ghana. Additionally, a larger nationwide population-based study is needed to provide much information about the foetal growth dynamics of Ghanaians.

CHAPTER ONE

1.0. INTRODUCTION

Live birth before 37 weeks of gestation, also known as preterm birth affects about 10% of all live births, totalling approximately 13.4 million births globally. Africa and South Asia contribute over 60% of these cases (WHO, 2018, 2023). It is a leading cause of neonatal mortality globally, responsible for nearly 1 million deaths annually (Liu *et al.,* 2016; WHO, 2018). In the Korle Bu Teaching Hospital, Ghana's largest tertiary facility, the prevalence is alarmingly high at 18.9% compared with 10% globally (Adu-Bonsaffoh *et al.,* 2019; WHO, 2023). An infant dies every hour within their first 30 days of life in Ghana due to preterm birth complications (Unicef Ghana, 2015). These complications also account for 16% of under-five child deaths and a staggering 35% of neonatal mortality worldwide (WHO, 2023). Numerous survivors encounter lifetime disabilities like cerebral palsy, visual impairment, hearing loss and low intellectual capabilities compared with infants born at term (Twilhaar *et al.,* 2019). These challenges are exacerbated in developing countries with limited healthcare resources. Preterm babies require significantly longer hospital stays, averaging 13 days compared to just 1.5 days for full-term infants, resulting in a more than seven-fold increase in hospital care costs (Castel *et al*., 2016; Purisch & Gyamfi-Bannerman, 2017).

Approximately two-thirds of preterm births occur spontaneously, driven by either spontaneous preterm labour or preterm premature rupture of membranes (PPROM) (Blencowe *et al.,* 2013). A smaller subset of preterm birth cases is provider-initiated due to maternal or foetal health concerns. The intricate nature of spontaneous preterm birth makes it difficult to comprehend, and available interventions remain limited. Its sudden occurrence poses significant risks to both the

expectant mother and her baby, as it often happens when they are unprepared for delivery (Rappoport *et al.,* 2018; Vogel *et al.,* 2018).

Epidemiological studies involving recurrent preterm births, elevated preterm delivery risk among women who themselves were born preterm, and twin studies suggest a genetic link to spontaneous preterm births (SPTBs) (Smid, *et al.,* 2017; Yang *et al.,* 2016). Women of African descent are postulated have 2 to 3-fold increased risk of SPTB compared to Caucasians, and over 5-fold risk for recurrent SPTB after adjusting for maternal environmental and socioeconomic status (Martin *et al.,* 2017; Smid, *et al.,* 2017). Some Genetic markers have been found to be associated with SPTB and gestational duration among Caucasians through genome-wide association studies (GWAS) (Huusko *et al.,* 2018; Zhang *et al.,* 2017). Other markers have also been found to increase the risk of preterm birth among African-Americans through candidate gene studies (Ferrand *et al.*, 2002; Frey *et al.,* 2016; Fujimoto *et al.*, 2002).

1.1. Problem statement

The aetiology of Preterm birth is complex, resulting from multiple biological, genetic, and environmental factors. Its complexity continues to hinder the development of appropriate diagnostics, neonatal care strategies, and interventions to prevent or manage preterm birth (Romero *et al.,* 2014). Preterm birth is a leading cause of new-born deaths worldwide, especially in low- and middle-income countries (LMIC). It strains neonatal units, increase healthcare costs, and exposes inequalities in maternal and neonatal health. Additionally, the prolonged hospital stays and special care needs for preterm neonates affects families financially, socially, and emotionally (WHO, 2023).

Research focused primarily on SPTB in Ghana is very limited, even though SPTB constitutes more than 60% of preterm births in Ghana. Thus, there is little to no empirical data on the aetiology, the clinical presentations, and the contribution of SPTB to overall preterm birth in Ghana, a country with a much higher prevalence of preterm birth compared to the global average.

Importantly, data on the genetic predisposition of Ghanaian women and African women in general to SPTB is virtually non-existent, even though, previous reports show a higher risk of African women to SPTB. Genetic markers identified to predispose Caucasians to SPTB cannot be reliable markers in Ghana to be exploited for interventions due to the genomic differences (Zaitlen *et al.,* 2017). Additionally, genetic markers associating African Americans to SPTB cannot equally be relied upon in Africa because the genetic similarity between African-Americans and Africans may be reduced by an average 20% due to genetic admixture (Zaitlen *et al.,* 2017). This is further exacerbated by increased genetic diversity among different African ethnicities (Bryc *et al.*, 2015).

The persistence of preterm birth, especially in LMIC continues to drive infant mortality upwards, increase health care cost, place emotional burden on families, and negatively affect the achievement of Sustainable Development Goal (SDG) 3 – ensuring healthy lives and promoting well-being for all ages (WHO, 2023).

1.2. Rationale

The study is timely because preterm birth continues to be a major global public health challenge as a leading contributor to neonatal mortality and lifetime disabilities (Liu *et al.*, 2016; Twilhaar *et al.*, 2019; WHO, 2023). The incidence and complications of preterm birth remain high,

especially in LMIC despite advances in maternal and neonatal care (WHO, 2023). Progress toward achieving SDG 3 targets for child survival requires urgent and evidence-based strategies, especially to minimise the occurrence of SPTBs (Vogel *et al.,* 2018). The study demonstrates the use of genetic analysis in identifying population-specific risk variants among Ghanaian women, an area currently under-researched.

By exploring the genetic factors influencing preterm birth, this study provides critical insights into the role genetics plays in preterm birth. It seeks to enhance the understanding of the biological pathways associated with preterm delivery, and highlights the need for the development of tailor-made preventive and therapeutic interventions to manage SPTB. Additionally, integration of genetic determinants with environmental and clinical risk factors in this research will produce findings which will strengthen existing theoretical frameworks on the multifactorial aetiology of preterm birth. This will provide additional strategies to inform current policy and intervention efforts.

A large European GWAS on SPTB has been conducted involving over 40 thousand participants Caucasian maternal samples to uncover genomic signatures of association with SPTB and gestational duration. This study found SNPs in *EARLY B-CELL FACTOR 1 (EBF1)*, *SELENOCYSTEINE-TRNA SPECIFIC (EEFSEC) and ANGIOTENSIN II RECEPTOR TYPE 2 (AGTR2),* genes to increase the risk of SPTB. In addition, SNPs in *EARLY B-CELL FACTOR 1 (EBF1)*, *SELENOCYSTEINE-TRNA SPECIFIC (EEFSEC)*, *ANGIOTENSIN II RECEPTOR TYPE 2 (AGTR2)* and *WNT FAMILY MEMBER 4 (WNT4)* genes were significantly associated with gestational duration, and SNPs in *ADENYLYL CYCLASE TYPE 5 (ADCY5)* and *RAP2C, MEMBER OF RAS ONCOGENE FAMILY (RAP2C)* had suggestive significant association with gestational duration at the genomewide level (Zhang et al., 2017). To the best of my knowledge,

no replication studies have been done on any of these novel genetic markers in Africa, particularly Ghana, to ascertain the association of these markers with risk of SPTB among Ghanaian women.

Furthermore, the *SERPIN FAMILY H MEMBER 1* (*SERPINHI*) (Wang *et al.,* 2006) and *MATRIX METALLOPROTEINASE 1* (*MMP1)* (Wang *et al.,* 2008) genes have been found to be associated with preterm premature rupture of membrane (PPROM), a subtype of SPTB among African Americans. The PPROM phenotype results from the disintegration of the foetal membrane. These two genes play critical roles in foetal membrane remodelling during pregnancy and parturition. Unequivocally, the sustenance of the uterine and cervix integrity via increased collagen synthesis as well as the regulation of *MMP1* expression to keep pregnancy to term is extremely crucial. As a result, population specific maternal genotyping of these genes to assess their association to SPTB is extremely crucial, particularly among women in African populations.

Thus, the genetics aspect of this study focused on the [-656]C >T SNP of *SERPIN FAMILY H MEMBER 1* (*SERPINHI*); *MATRIX METALLOPROTEINASE 1 MMP1*; *EUKARYOTIC ELONGATION FACTOR, SELENOCYSTEINE-TRNA SPECIFIC* (*EEFSEC*); *ANGIOTENSIN II RECEPTOR TYPE 2* (*AGTR2*); *WNT FAMILY MEMBER 4* (*WNT4*) and *ADENYLYL CYCLASE TYPE* 5 (*ADCY5*) genes and SPTB among Ghanaian women.

1.3. Hypothesis

The risk of SPTB in Ghanaian women is modulated by specific clinical presentations and variants at specific gene loci.

1.4. Main objective

This project sought to identify peculiar phenotypes and genetic variants associated with SPTB among Ghanaian women.

1.5. Specific objectives

Specific objective 1: To describe clinical presentations associated with SPTB among Ghanaian women and their neonates.

Specific objective 2: To identify genetic markers underpinning of SPTB in Ghanaian women.

Specific objective 3: To characterize effect of *SERPINH1* -656 C/T genotype on placental tissue.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Parturition (childbirth)

Parturition (childbirth) is a process starting from fertilization, implantation in the uterus, resulting in embryonic and foetal growth and ending in the spontaneous expulsion of the foetus through the vagina or assisted delivery via caesarean section. During this period, the maternal body goes through significant anatomical and physiological changes to support the developing foetus (Romero, 2021). These include hormonal shifts, alterations in the uterus, cervix and maternal physiology (Menon *et al.,* 2020). It normally takes an approximately 40-week duration. Reduced gestational periods, especially those less than 37 weeks pose significant health risks to the baby due to immaturity of their organs to support their survival in the extrauterine environment (Beck *et al.,* 2010).

2.2. Biological pathway to spontaneous birth

The progression to spontaneous birth can be grouped into four phases. These are: Phase 0 (quiescence), Phase 1 (activation), Phase 2 (stimulation), Phase 3 (involution) as indicated in **Figure**. **2.1**.

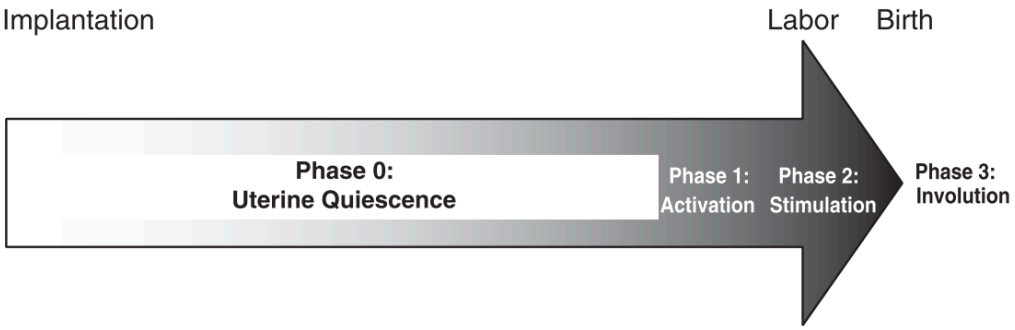


Figure 2.1: Stages of parturition. *The four-phase parturition process, starting from implantation, involves quiescence, making up over 9 out of 10 of the entire duration of parturition. This proceeds into the stage of activation, influenced by increased expression of contraction-associated proteins receptors. The next phase, stimulation, involves a cascade of events triggered by myometrial activation, resulting in events like cervical ripening. In the involution phase, there is placenta separation and uterine contraction, principally influenced by maternal oxytocin. The diagram was obtained from "Preterm Birth: Causes, Consequences, and Prevention" (Behrman & Butler, 2007).*

Phase 0 is the state of uterine quiescence (inactivity). It constitutes about 95% of the gestation period. Myometrial activity is regulated by biochemical mediators like progesterone, prostacyclin, relaxin, and nitric oxide (Behrman & Butler, 2007). Occasionally, weak and uncoordinated Braxton-Hicks contractions may occur due to reduced gap junction protein expression, resulting in fewer synchronized contractions in the pregnant myometrium (a layer of smooth muscle tissue that makes up the bulk of the uterine wall) (Garfield *et al.,* 1992).

Phase 1 involves biochemical alteration of uterine myometrium encompassing the expression of contraction-associated proteins (CAPs) like connexin-43, oxytocin and prostaglandin receptors. This phase is initiated by uterine stretch resulting from foetal growth or the activation of the foetal hypothalamus-pituitary-adrenal (HPA) axis due to foetal maturation, or both (Behrman & Butler, 2007; Di Renzo *et al.,* 2018)

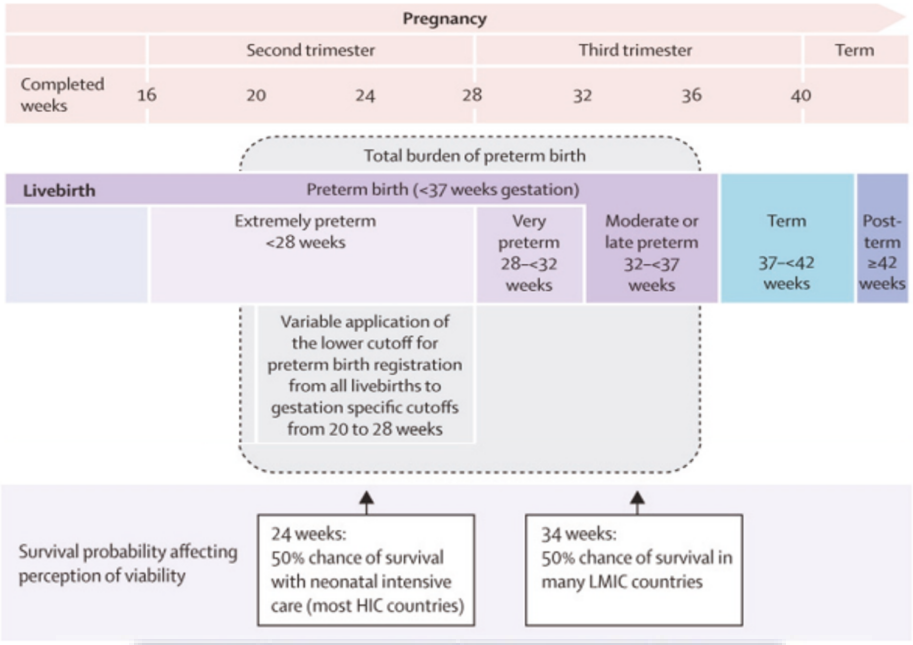
Phase 2 proceeds from the priming of the uterus in phase 1 for contraction to occur. There are progressive events in the labour process via uterine contractions, cervical ripening, and foetal membrane activation. Cervical ripening involves the gradual remodelling of the cervix extracellular matrix via reduced total collagen content, increased collagen solubility and collagenolytic activity (Gilman-Sachs *et al.,* 2018). Moreover, a surge in placental corticotropin releasing hormone (CRH) production results in functional progesterone withdrawal in the myometrium. This reduces the physiologic impact of progesterone and activates the quiescent myometrium. (Cappelletti *et al.,* 2016). The resultant effect is an increase in prostaglandin production which also activates matrix metalloproteinase (*MMP*) genes leading to catabolism of structural proteins such as collagen and elastin. This allows cells to modify and remodel the extracellular matrix in the cervix and decidua (a specialized tissue that lines uterus which nourishes and support the developing embryo and foetus). Conversely, matrix metalloproteinases *MMPs* could also be triggered by an invasion of immune cells such as neutrophils and macrophages (Osman *et al.,* 2003; Payne *et al.,* 2012). In addition, there is a proliferation of inflammatory mediators including cytokines like Interleukin-1(IL-1), Tumour Necrosis Factor-alpha, Interleukin-6 (IL-6) and Interleukin-8 (IL-8) (Dubicke *et al.,* 2010, 2016). These activities promote cervical ripening and decidual and foetal membrane activation. Decidual and foetal membrane activation is a process resulting in the separation of the foetal membranes made up of the amnion (inner membrane) and the chorion (outer membrane) located at the lower part of the uterus (lower pole membranes) from uterine decidua, culminating in spontaneous membrane rupture. Additionally, mechanical stretch from foetal growth mounts pressure on the cervix. It also reduces blood supply, resulting in increased hypoxia and hypoxia-inducible factor-1a (HIF-

1a) in the cervix which advances softening and dilation of the cervix through inflammation via a feed-forward process (Kishore et al., 2012; Yellon, 2017, 2020).

Phase 3 encompasses placental separation and uterine contraction. The placenta separates by cleaving along the decidua basalis (the maternal part of the placenta). Increased uterine contractions reduces bleeding from exposed venous sinuses that are exposed after delivery of the placenta, and are mainly regulated by oxytocin (Behrman & Butler, 2007). It is hypothesized that preterm and term labour share a common pathway with interactions between pathologic and physiological factors, especially after 32 weeks. Preterm labour is triggered by pathologic processes, while term labour by physiological activation (Cappelletti *et al.,* 2016).

2.3. What is preterm birth?

The World Health Organization (WHO) defines preterm as all births before 37 completed weeks of gestation or before the 259th day since the first day of a woman's last menstrual period (WHO, 1977). The 37-week cut-off for preterm birth is considered arbitrary because lower gestational age increases risks to the unborn baby. Even babies at 37 or 38 weeks face higher risks compared with those born at 40 weeks (Marlow, 2012). The gestational age can be sub-divided into extremely preterm (less than 28 weeks' gestation) constituting about 5% of all preterm births; very preterm (28–31 weeks' gestation), about 15%; and moderate to late preterm which constitute about 80% (Blencowe *et al.,* 2013). The preterm subtypes are exhibited in **Figure 2.2.**



Key: HIC = high income countries; LMIC = Lower-to middle-income countries

Figure 2.2: Overview of preterm birth definition. *Livebirth before 37 weeks' gestation is further categorised based on gestational age into: extremely preterm, very preterm and moderate to late preterm. The chance of survival decreases with gestational age. However, survival is much higher in high income countries HIC compared with lower to middle income countries (LMIC). The diagram is a modified diagram initially obtained from "Born Too Soon: The global epidemiology of 15 million preterm births" (Blencowe et al., 2013).*

2.4. Public health burden of preterm birth

Preterm results in neonatal complications due to immaturity of multiple organ systems as well as neurodevelopmental disorders. It is estimated to be responsible for 35% of the world's 3.1 million annual neonatal mortalities and second to pneumonia as the major cause of child mortality under 5 years old (WHO, 2023). About half the number of babies born at or below 32 weeks die in developing countries while over 90% of these survive in developed countries (WHO, 2023). Preterm birth complications affects the entire life cycle and contributes significantly to number of years lost as a result of ill health, disability or early death (WHO, 2023). Children born preterm are approximately 80 times and 10 times more likely to develop

cerebral palsy and mental retardation respectively. Additionally, children born preterm are 20 times more likely to develop major disabilities such as: visual impairment, hearing loss compared with infants born at term (Moster *et al.,* 2008).

Preterm birth also imposes a significant psychological stress on families with preterm children. A 13-year longitudinal study found that parents with 7-year old very preterm children experienced an 18-fold risk of parental stress after adjusting for social risk and child disability (OR = 18.2, 95% CI: [6.3 – 30.1], p = 0.03) and 22.5-fold risk of parental stress after adjusting for child neuro-developmental disability (OR = 22.5, 95% CI: [10.5 – 34.4], p < 0.001) compared with mothers with term children (Treyvaud *et al.,* 2014). Another research conducted by Yaari and colleagues observed that mothers with preterm children exhibited a consistent psychological distress over a 13-year period compared with of mothers full-term children (Yaari *et al.,* 2019).

Colossal national and family income is spent to take care of preterm children. The United States for example spends in excess of $25 billion annually, almost $65,000 per preterm birth relative to term or post-term (pregnancy beyond 42 gestation weeks) births. The cost of care increases by 12-fold from $28,367 at preterm occurring between 32 and 36 gestation weeks to $344,355 for children born at less than 28 gestation weeks (Waitzman *et al.,* 2021). In Australia, the cost increases in excess of 9-fold from $25,417 at preterm occurring between 32 and 36 gestation weeks to $236,036 for children born at less than 28 gestation weeks (Newnham *et al.,* 2022). Unfortunately, data on the economics of preterm birth in developing countries like Ghana is very scanty.

2.5. Aetiologies of Preterm Birth

Preterm birth is grouped into spontaneous preterm birth (SPTB) and medically-indicated preterm birth, also called provider-initiated preterm. The medically mediated involves the induction of labour or elective caesarean birth before 37 weeks of gestation in situations such as severe preeclampsia, uterine rupture and foetal growth restriction when the risks to the foetus or mother is higher than the benefit of continuing the pregnancy (Harrison *et al.,* 2016).

SPTB on the other hand is the "unintentional and unplanned delivery before 259 days of gestation" (NICHD, 2023). The cause of SPTB is not well understood. However, SPTB is often regarded as an inflammatory process due to the role inflammatory and immune responses play in the complex pathways leading to the onset of SPTB. In the context of preterm birth, inflammation involves the activation of immune cells, release of inflammatory molecules, as well as the disrupts the delicate balance that maintains pregnancy until full term (Tantengco *et al.,* 2021). Nonetheless, even though inflammation is recognized as a major contributor to SPTB, not all cases of SPTB are solely due to inflammation (Yellon, 2017, 2020). Multiple pathways and factors are involved in the complex process of SPTB. As a result, the interplay between inflammation, hormonal changes, mechanical stress, and genetic factors are being elucidated. About 70% of preterm birth prevalence is SPTB. It is sub-categorised into spontaneous preterm labour with intact membrane and preterm premature rupture of membranes (NICHD, 2023).

2.6. Spontaneous preterm labour with intact membrane (SPTL)

It is the regular active contractions of the quiescent myometrium "occurring at a frequency of at least two every 10 minutes associated with cervical ripening that lead to the dilation and effacement of the cervix before 37 completed weeks of gestation and require hospitalization"

(Romero *et al.,* 2010). It constitutes about two-thirds of SPTBs (American College of Obstetricians and Gynecologists, 2007). The exact cause of spontaneous preterm labour with intact membranes remains unclear. However, it is attributed to the interplay of factors including infection and cervical ripening. (Romero *et al.,* 2014). Another attributable factor is antepartum haemorrhage, which is the vaginal bleeding that occurs after the 20th week of pregnancy but before the onset of labour. It is also associated with placenta previa (placenta partially or completely covering the cervix) and placental abruption, also known as abruptio placentae (the placenta detaches from the uterine wall before delivery) resulting in bleeding between the placenta and the uterine wall. Other factors observed to result in SPTL include: psychological stress, and idiopathic (unknown) contraction. (Rappoport *et al.,* 2018; Strauss e*t al.,* 2018). Additionally, increased expression of myometrium transmembrane protein, connexin (CX)-43 has been associated with SPTL (Singh *et al.,* 2019). The CX-43 contributes to the responsiveness of myometrial cells to oxytocin, facilitates their spread and promoting coordinated contractions. CX-43 is also implicated in the process of cervical ripening, by transmitting and amplifying inflammatory signals such as prostaglandins, IL-8, IL-1 and MMPs (Singh *et al.,* 2019).

2.7. Premature prelabour rupture of membrane (PPROM)

It is the spontaneous rupture of the foetal membranes (amnion and chorion) before 37 gestation weeks, at least 1 h before the onset of contractions (Welzing *et al.,* 2011). The foetal membranes rest on a collagenous basement membrane which provides substantial structural strength for the membranes. Membrane rupture involves extracellular matrix remodelling via collagen degradation. This is mainly undertaken by matrix metalloproteinases (MMPs) which are also regulated by Tissue Inhibitors of Metalloproteinases (TIMPs) (Jang *et al.,* 2022; Nguyen *et al.,*

2023). PPROM is a complex and multifactorial condition influenced by various genetic, environmental and hormonal factors. It has generally been attributed to other pathophysiological events such as apoptosis-induced degradation of the extracellular matrix. (Fortunato & Menon, 2001; Kovács *et al.,* 2020; Negara *et al.,* 2018, 2020).

It is also triggered by stretch-induced physical weakening of the foetal membranes initiated by polyhydramnios (excessive amniotic fluid accumulation in sac around developing foetus) and multi-foetal gestations (Barrett *et al.,* 2019). A common attributable risk factor is asymptomatic intrauterine infection with pathogens transmitted to the uterus through ascending infection and haematogenous routes that increase proteolytic enzymes activity (Choudhary *et al.,* 2015; Vinturache *et al.,* 2016). An analysis of data reveals that while 4% of PPROM occurs in subsequent pregnancies following term deliveries without PROM complications, the likelihood rises to 21% if the initial pregnancy involved preterm PROM (Flood & Naeye, 1984).

2.8. Phenotypic characterization of SPTB

SPTB is a complex condition, but likely to have an ultimate pathway linked with multiple causes. As a result, women with similar SPTB causes could be grouped to provide plausible inferences (Manuck *et al.,* 2015). PPROM and SPTL are described as phenotypes because of their distinct characteristics (Manuck *et al.,* 2015). Some phenotypes identified include: systemic and intrauterine infection/inflammation (Hills *et al.,* 2022; Lye *et al.,* 2021; Tantengco & Menon, 2021), uteroplacental thrombosis (blood clots formation within the uteroplacental blood vessels) (Salafia *et al.,* 1995), intrauterine vascular lesions (pathologic changes in the blood vessels of the uterus) and decidual haemorrhage (bleeding or the accumulation of blood within the inner lining of the uterus) (Ericksen *et al.,* 2020; Paz-Levy *et al.,* 2017). Other phenotypes include pathologic

uterine overdistension (excessive stretching and enlargement of the uterus beyond its normal capacity) (Behrman & Butler, 2007) and maternal stress (Bergeron *et al.,* 2023; Burris *et al.,* 2020; Kornfield *et al.,* 2022)

Some researchers have also characterised SPTB based on genetic or familial or intergenerational factors (Huusko *et al.,* 2018; Zhang *et al.,* 2018). Others also characterise SPTB based on maternal age (< 19 years and > 35 years) (Marvin-Dowle *et al.,* 2018; Tembo *et al.,* 2020; Wallace, 2019), anaemia (Finkelstein *et al.,* 2020; Kumari *et al.,* 2019; Zhang *et al.,* 2009), nutrition (Bloomfield *et al.,* 2003) and early menarche and many other attributable risk factors (Li *et al.,* 2017).

2.9. Maternal age and SPTB

Women go through several developmental changes in their growth process that predispose them to giving birth to children with neonatal disorders as well as preterm birth. Some studies suggest that teenage mothers are not anatomically well developed to handle foetuses. According to Oxlund and colleagues (2010), collagen concentration and its stiffness in the cervix increases with age. Collagen is the major constituent of the cervix. It is thus possible that the collagen content of the cervix of adolescents is not adequate in composition and characteristics compared with adults to keep the strength of the cervix to sustain pregnancy to term (Oxlund *et al.*, 2010). Series of animal experiments conducted by Wallace and colleagues using adolescent pregnant sheep demonstrated increased adolescent's maternal growth at the expense of foetal resulting in compromised foetal nutrition and preterm birth (Wallace, 2019; Wallace *et al.,* 1996, 1999, 2010). Nonetheless, to deduce whether confounding factors inherent in teenage pregnancies or maternal and/or foetal nutrition play a role in the high risk of preterm birth among teenage

pregnancies is still a difficult task (Bloomfield, 2011). Other studies have also attributed the increased prevalence of preterm delivery among adolescents to lifestyle behaviours like smoking and psychological stress which have independently been found to be associated with preterm birth (Delcroix *et al.*, 2023)

Smith and Pell (2001) studied 110,233 deliveries in Scotland, and found an association between second births among ages 15-19 and increased preterm birth risk "OR = 1.6, 95% CI: [1.2 – 2.1)" and extreme preterm almost a three-fold OR = 2.5, 95% CI: [1.5 to 4.3] (Smith & Pell, 2001). In another population-based study, Hammond *et al.* (2013) characterised risk factors of 526,125 SPTBs birth in Western Australia and found age < 20 as a risk factor for SPTL, OR = 1.27, 95% CI: [1.16 – 1.38] but protective against PPROM, OR = 0.84, 95% CI: [0.73 – 0.94]. However, maternal age ≥ 35 was a risk factor for both SPTL, OR = 1.30, 95% CI: [1.22 – 1.39] and PPROM, OR = 1.92, 95% CI: [1.76 – 2.08]. Several population-based studies have also found an association between maternal age of less than 20 and SPTB (Bakker et al., 2011; Khashan *et al.,* 2010; Marvin-Dowle *et al.,* 2018; Tembo *et al.,* 2020).

However, maternal age greater than 35 years has also been found to be associated with either SPTB or medically indicated preterm births. Increasing maternal age predispose them to noncommunicable diseases like obesity, hypertension and diabetes that trigger inflammatory processes which predispose them to preterm birth in general (Lynch *et al.,* 2014). Type 2 diabetes can activate inflammatory signalling pathways like the nuclear NF-κB pathway. High insulin levels can also contribute to oxidative stress, which can damage cells and tissues, triggering an inflammatory response. These lead to the production of pro-inflammatory molecules that could trigger SPTB via cervical ripening, and decidual and foetal membrane activation (South *et al.,* 2019).

Waldenström and colleagues conducted a population-based study on the Swedish Medical Birth Register involving 2,009,068 participants and found preterm birth risk to increases with increasing age above 30 years, irrespective of parity. Adjusted odds ranged from: 1.18 to 1.28, 1.59 to 1.70 and 1.97 to 2.40 at ages 30–34 years, 35–39 years and ≥ 40 years, respectively (Waldenström *et al.*, 2017). A similar result was obtained by Zapata-Masias and colleagues (Zapata-Masias *et al.*, 2016). Nonetheless, some studies find association between maternal age and preterm birth have produced mixed results. A Chilean study involving 4,956,311 births found age ≤ 19 years to be protective against all preterm birth subtypes. However, age >35 years was a risk factors for all preterm birth subtypes. Factors such as multiple gestations, and chronic pathologies like gestational hypertension, pre-eclampsia and eclampsia were identified (Araya *et al.,* 2017).

2.10. Maternal psychological distress and SPTB

Psychological distress encompasses negative emotional states arising from stressors, challenges, or internal worries. Psychological distress could be expressed in the form of: stress (natural and adaptive reaction that prepares the body to cope with perceived threats or demands often related to specific situations or events); anxiety (internal worries, fears, or apprehensions about potential future events characterized by persistent unease, nervousness, and excessive worry, and often generalized rather than tied to a specific situation); and depression (persistent low mood, lack of interest or pleasure in activities, and a sense of hopelessness) (Schetter, 2009). Chronic maternal stress predispose them to mental health conditions such as major depressive and anxiety disorders that affect their wellbeing (Szegda *et al.,* 2014).

Increased maternal psychological stress levels amplify the hypothalamic-pituitary-adrenal (HPA) axis activity that leads to increased maternal cortisol production in a dose-response manner. This results in a cumulative physiological toll of chronic stress known as maternal allostatic load (Christiaens *et al.,* 2015). The cortisol activates glucocorticoid receptors (GRs) and interact with glucocorticoid response elements (GREs) at the promoter regions of target genes like *CYCLOOXEGENASE-2* (*COX-2*) resulting in the production of prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) via prostaglandin H_2 (PGH_2). These end products stimulate uterine contractions by increasing the sensitivity of uterine muscle cells to calcium ions (Lee *et al.,* 2017).

The association between maternal prenatal psychological stress and preterm birth has been carried out by several researchers. In a Swedish longitudinal study of 2,618,777, Class and colleagues found that maternal stress exposure resulting from death of the father of the child or first-degree relative during mid-gestation (5^{th} and 6^{th} month) was associated with the heightened risk for shortened gestational age (-0.52 days, standard error = 0.15, p = .0006) and the greatest risk of preterm birth OR = 1.24, 99% CI: [1.08 -1.42] (Class *et al.*, 2011).

Intimate partner violence (IPV) is among the most widespread global public health issues, and has dire impacts on expectant mothers (García-Moreno *et al.,* 2013). Martín-de-las-Heras and colleagues established from a cohort of 779 mothers in Spain that intimate partner violence was associated with over 2-fold risk of preterm birth (aOR = 2.4, 95% CI: [1.1 – 5.0], p = 0.01) (Martín-de-las-Heras *et al.,* 2022). A prospective cohort study involving 1112 pregnant women in Moshi–Tanzania also observed a 3-fold increased association between physical IPV and preterm birth (aOR = 2.9, CI: 95% [1.3 – 6.5]. A similar observation has been made in other studies in Zimbabwe (Sigalla *et al.,* 2017; Yaya *et al.,* 2021). Work related stress such as

standing for longer hours could reduce blood circulation to the uterus and may result in venous congestion and increased pressure in the pelvic region; mechanical stress placed on the uterine muscles and the cervix could trigger preterm labour if prolonged (Strohmaier *et al.,* 2019). A population-based case–control study in USA found that increased 'occupational physical activity' increased the risk of preterm birth by 24%, (aOR = 1.24, 95% CI: [0.93 – 1.64]; p for trend=0.01) (Lee *et al.,* 2017). Niedhammer *et al.* (2009) also found any of the following two factors: work contract, working hours, shift work and physical demands to increase the risk of preterm delivery by over 5-fold (Niedhammer *et al.,* 2009).

Studies have found genetic and epigenetic association between prenatal stress and preterm birth. Vidal and colleagues explored the DNA methylation at imprinted genes *H19*, *IGF2*, *MEG3*, *MEST*, *SGCE*, *PEG10*, *PEG3*, *NNAT*, and *PLAGL1* to investigate the link between prenatal stress and preterm birth (PTB) in 537 pregnant women. They found no association (aOR = 0.98, [95% CI: [0.4 – 2.40]; $p < 0.96$) after adjusting for maternal BMI and blood pressure. Rather, maternal stress was associated with increased DNA methylation at the *MEST* gene's differentially methylated region (DMR) in infants of the mothers (2.8% difference, $p < 0.01$) (Vidal *et al.,* 2014). The *MEST* gene expression is associated with obesity. Thus, providing information on how prenatal stress influences offspring's epigenomic and non-communicable diseases (El Hajj *et al.,* 2013).

2.10.1. The CES-D scale for Maternal Depression screening

The Centre for Epidemiological Studies-Depression (CES-D) is used to measure the psychological state of an individual in the 7 days prior to the interview. It is utilised as a screening procedure for onward psychological assessment (Radloff, 1977). The CES-D scale

comprises 20 questions and responses used to evaluate the perceptions and behaviours of the respondent. Four of the questions (4, 8, 12, and 16) solicit the "positive perception" of the respondents. The responses are scored from 3 to 0, and the score of 3 denotes that those "perceptions" rarely happened (maximum, 1 day) and 0 indicates that those "perceptions" occurred most of the days (5 to 7 days) of the week. The remaining questions solicit the occurrence of "negative perceptions" from the respondent. The scores are reversed in such circumstances, thus 0 to 3. The scores are then summated, with the highest score being 60. The scale items and the scoring procedure for the CES-D scale are shown in **Table BI** and **Table BII** of the **Appendix B**. A score of 16 and above indicates depression, a score lower than 16 suggests no depression. A more detailed categorisation involves segregating the scores into four domains namely: "No depression" (CES-D score <16), "Possible depression" ($16 \geq$ CES-D score ≤ 19), "Depression" ($20 \geq$ CES-D score ≤ 24), and "Severe depression" (CES-D score ≥ 25) (Wemakor & Mensah, 2016).

2.11. Age at menarche and SPTB

Menarche is the first occurrence of menstrual bleeding, an indicator of puberty and typically occurring as a result of hormonal changes, especially increased production of sex hormones, particularly oestrogen (Hamajima *et al.,* 2012). The onset of menarche is a polygenic trait and is epidemiologically linked to various diseases in adulthood. Early menarche has been linked to several adverse health risks such as cancer (Day *et al.,* 2017; Hamajima *et al.,* 2012; Song *et al.,* 2022; Yang *et al.,* 2022), type 2 diabetes (Cheng *et al.,* 2020; Elks *et al.,* 2013) and cardiovascular diseases (Bubach *et al.,* 2021). A hormone at the prepubertal stage found to activate the onset of menarche is oestradiol which is essential for the expression of kisspeptins.

This is a protein encoded by the *KISS1* gene which activates the gonadotropin releasing hormone (GnRH) neurons to release GnRH. This then stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which also regulate the development of the gonads, production of sex hormones and the onset of puberty resulting in menarche in females (Karapanou & Papadimitriou, 2010).

Increased prepubertal production of oestradiol has been associated with early onset menarche. Additionally, women with early menarche, usually those with menarche latest at 12 years are subject to an early and a life-long high degree of oestradiol stimulation. As a result, biochemical pathways harmful to the health of women with such physiology are unduly regulated (Apter *et al.,* 1989). In a study of 204 Norwegian women, Emaus and colleagues observed an almost 4-fold increase in salivary 17-beta-estradiol among those with age at menarche ≤12 years (3.7 pmol/l (95% confidence interval, 1.8-5.7 pmol/l) (Emaus *et al.,* 2008). Apter and colleagies made a similar observation earlier (Apter *et al.,* 1989).

Increased serum oestradiol triggers the expression of oxytocin receptor, prostaglandin receptors and stimulates prostaglandin synthesis via the synthesis of COX-2. These processes further quicken myometrial contractility and labour (Behrman & Butler, 2007). A couple of studies have recorded amplified concentrations of oestradiol in the body fluids of women with preterm births compared with those with term births. A study of 17β-oestradiol and progesterone concentrations in the amniotic fluid and plasma of 40 women with singleton observed a significantly higher median amniotic fluid β-oestradiol concentration (1.5 ng/ml vs 0.9 ng/ml, p = 0.0001) and plasma β-oestradiol concentration (14.1 ng/ml vs 6.9 ng/ml, p = 0.022) among women with preterm deliveries compared with those with term deliveries. Additionally, they observed a significantly lower median amniotic fluid progesterone/17β-oestradiol (18.4 vs 33.6,

respectively, p = 0.0017) and plasma (9.8 vs 17.0, respectively, p = 0.016) concentrations than those with term deliveries (Mazor *et al.,* 1994). Li and colleagues discovered from 11,016 Chinese women that earlier menarche (≤11 years) was associated with preterm birth occurrence, OR 1.67 (95% CI: 1.18-2.36) when 13 years was used as the reference age (Li *et al.,* 2017). Early onset of menarche is also associated with depression, a risk factor of SPTB (Shen *et al.,* 2019). Late onset of menarche has also been found to be protective against SPTB. In a case-control study involving 8264 in the Boston Birth Cohort, a year later menarche onset of menarche decreased the odds of preterm birth 5% (95% CI: 2% - 8%), after adjusting for variables including maternal birth place, smoking status and pre-pregnancy BMI (Chen *et al.,* 2023). Similarly, a Japanese cohort study of 37 645 singleton pregnancies observed late onset of menarche (≥15 years) to be protective against the risks of preterm birth RR = 0.79, 95% CI: [0.64–0.98], using age 12 as reference age. However, they did not find an association between early age at menarche (≤9 years) and preterm birth (RR = 1.10, 95% CI: [0.70–1.72]) (Kanno *et al.,* 2022).

2.12. Parity and SPTB

Parity is determined by counting the number of instances in which a woman has delivered a live neonate (at any gestation) or a foetus at 24 weeks or beyond, without distinguishing between viable and non-viable outcomes (Maraj & Kumari, 2021). Parity is classified into nulliparity, primiparity, multiparity and grand multiparity. Nulliparity results when a woman who has never given birth and is experiencing her first pregnancy. Primiparity is a situation when a woman has given birth once. Two or more birth by a woman results in multiparity. However, multiparity is classified into 2 to 4 births which is called multiparity and 5 or more births classified as grand

multiparity. They all have their ramifications regarding the occurrence of SPTB (Kozuki *et al.,* 2013).

The association between parity and SPTB appears to be U-shaped. A Dutch study involving 802,119 pregnancies, identified a 95% increased risk associated with SPTB birth in nulliparous women, OR 1.95, 95% CI: [1.89–2.00] and 26% increased risk multiparous women (OR = 1.26, 95% CI: [1.13–1.41]) compared to women with 2 pregnancies (Koullali *et al.,* 2020). Similarly, a retrospective study of 784 singleton preterm births in Nigeria also found nulliparity as an independent risk factor for preterm birth by 2-fold (aOR = 2.08, 95% CI: [1.22, 4.91]) (Iyoke *et al.,* 2015). Some population-based studies (Ananth *et al.,* 2007; Delnord *et al.,* 2018) and meta-analysis (Kozuki *et al.,* 2013) have made similar observations.

The increased risk of SPTB among nulliparous women has been attributed to non-available birth history which makes it difficult for health care providers to provide the appropriate attention (Koullali *et al.,* 2020). Additionally, inexperience in pregnancy matters among nulliparous women do not make them seek the right medical attention (Govender *et al.,* 2020; Wiemann *et al.,* 2005). Moreover, maternal biological characteristics such as smaller uterine cavity, especially among the teenage nulliparous women predispose them to the condition (Canteiro et *al.,* 2010; Wildemeersch *et al.,* 2013). Furthermore, nulliparous women are more predisposed to uterine artery notches which is associated with heightened uteroplacental blood impedance resulting in diminished uteroplacental perfusion and a resultant reduction in nutrient and oxygen supply to the foetus (Derwig *et al.,* 2013; Derwig *et al.,* 2011). A uterine artery notch is a feature observed in Doppler ultrasound studies, a non-invasive imaging technique relying on sound waves to study the blood flow of the uterine arteries during pregnancy (Hernandez-Andrade *et al.,* 2002). However, women with increasing parity are predominantly older and predisposed to

medical complications including hypertension and diabetes, placental pathologies such as placenta previa and placental abruption, and cervical incontinence that predispose them to SPTB (Aliyu *et al.,* 2005). Kozuki *et al.,* observed in study that parity greater than 3 with maternal age also greater than 35 years increases the risk of SPTB by 43% (aOR = 1.43, 95% CI: [1.21-1.69]) as well as parity greater than 3 with maternal age ranging between 17 and 35 years (aOR =1.20, 95% CI: [1.06-1.35]).

2.13. Marital status and SPTB

Relationships play a vital role in contributing to health and overall well-being. Social isolation represents a substantial risk factor for morbidity and mortality (Becker *et al.,* 2019; Sassler & Lichter, 2020; Umberson & Thomeer, 2020). For many adults, a pivotal relationship is marriage. Ideally, it is an avenue for a healthy environment pre-requisite for procreation (Campbell *et al.,* 2023). Unmarried women especially, the youth are prone to lifestyle habits like smoking, excessive alcohol drinking (Guo *et al.,* 2019) and extreme psychological stresses due to relationship uncertainties (Nyadanu *et al.,* 2022) which are attributable risk factors for SPTB (Martín-de-las-Heras *et al.,* 2022).

A retrospective of singleton preterm of 784 singleton births in Nigeria found that being unmarried was an independent risk factor for preterm birth by almost 2.5-fold, (aOR = 2.41, 95% CI: [1.56 - 3.71]) (Iyoke *et al.,* 2015). Similarly a case-control study in Malaysia involving 1,559 women found unmarried women to be almost 4-fold associated with increased risk of SPTB, OR 3.81 = 95% CI:[1.78 - 8.13]) (Tan *et al.,* 2017). A similar observation was earlier made by Zeitlin and colleague (Zeitlin *et al.,* 2002). Marriages offer the opportunity for paternal support during pregnancy. Paternal presence has been shown to have a protective effect and serve as

proxy measure for paternal support. Using the Virginia birth registry data (*N* = 101,602), Masho and colleagues found that unmarried women with no paternity increased the risk of preterm birth by 60%, OR = 1.57, 95% CI: [1.41 – 1.75]) (Masho *et al.,* 2010). In a similar research, Surkan and colleagues observed from the Boston Birth Cohort (N = 7,047) that paternal absence was associated with 21% higher risk of preterm birth (OR 1.21, 95% CI: [1.01–1.45]) (Surkan *et al.,* 2017).

Systematic reviews and meta-analysis have also found associations between marital status and SPTB. Shah and colleagues in systematic reviews and meta-analysis of 21 studies found that being unmarried was associated with increased risk of preterm birth by 22%, OR = 1.22 (95%CI: [1.14 – 1.31]), being single 54%, OR = 1.54 (95%CI: [1.39 – 1.72]) and cohabitating 15%, OR = 1.15, 95%CI: [1.08 – 1.23]) (Shah *et al.,* 2011).

2.14. Antenatal visits and SPTB

Antenatal care (ANC) are designed to: monitor the health of both the mother and the baby, detect and prevent potential complications, and provide essential information and support for a safe pregnancy and childbirth (WHO, 2016). ANC is crucial, offering health promotion, screening, and support (WHO, 2016). The WHO has a comprehensive guideline on routine ANC which focuses on person-centred health and well-being beyond mortality prevention. Informed by women's views, the guideline emphasizes a positive pregnancy experience, including maintaining normality, ensuring a healthy pregnancy, effective transition to labour, and fostering positive motherhood. The recommendations respond to the complexities of ANC practice, emphasizing holistic care to enhance the overall well-being of pregnant women and adolescent girls (WHO, 2016).

According to the Ghana Demographic and Health Survey (GDHS), 2022, Ghana has an antenatal coverage of 98% for at least one ANC visit during pregnancy. However, 88% of Ghanaian pregnant women attended at least four ANC visits before delivery (GSS GHS and ICF, 2023). The WHO recommended a minimum of eight ANC visits for a healthy pregnancy (WHO, 2016). However, 4 and above is regarded as a good attempt and less than 4, a poor attempt for quality health care service (Adu-Bonsaffoh *et al.,* 2019; Tuladhar & Dhakal, 2012). Poor ANC visit has implications on the unborn baby and the expectant mother, and associated with increased neonatal and maternal morbidity and mortality (Belachew *et al.,* 2022; Boafor *et al.,* 2021; Upadhyay *et al.,* 2019). Many adverse pregnancy outcomes including SPTB have also been associated with poor ANC attendance.

A retrospective study of 7801 singleton births in Ghana found poor ANC visits to be independently associated with increased preterm birth risk by more than 5-fold, aOR = 5.17 (95% CI: 4.057–6.594, $p < 0.001$) (Adu-Bonsaffoh *et al.,* 2019). Another study of 390 mothers in Ghana by Aseidu and colleagues found ANC attendance of four or more visits to be independently associated with preterm birth reduction by 80% (aOR = 0.2; 95% CI: 0.1–0.4) (Aseidu *et al.,* 2019). Similarly, Tan and colleagues identified an almost 3-fold, OR 2.62 = (95% CI: 1.14-5.99) increased association between poor antenatal visit and SPTB (Tan *et al.,* 2017).

The timing of the visits is also very important. Antenatal visits initiation from the first trimester is recommended and reduces adverse birth outcomes compared with initiation from the second trimester onwards (Beeckman *et al.,* 2013). Receiving all interventions beginning from thew first trimester is associated with the risk of preterm birth risk reduction by almost 80%, OR = 0.21, 95% CI: [0.06 – 0.68]) (Beeckman *et al.,* 2013) and 2-folds (Sarker *et al.,* 2020).

2.15. Maternal HB at booking and SPTB

The four-subunit-protein complex with a heme group, called haemoglobin (Hb), located in the red blood cells, transports oxygen throughout the body. Additionally, it is capable of transporting carbon (IV) oxide from other body tissues to the lungs for exhalation, as well as bind to hydrogen ions (H^+) to help maintain blood pH (Berg *et al.*, 2002). Hb levels for diagnoses of anaemia is based on the pressure oxygen exerts (partial pressure of oxygen [pO_2]) at sea level (21.2 kilopascals [kPa]) which is a part of the atmospheric pressure (760 mmHg or 1 atmosphere (atm) or 101.325 kilopascals (kPa). Increasing altitude reduces atmospheric pressure and pO_2, a phenomenon resulting in reduced oxygen molecules per volume of air which influences the body to escalate Hb synthesis to compensate for the reduced oxygen availability (Berg *et al.*, 2002).

A precise definition of anaemia during pregnancy is confounded by various factors such as altitude, use of iron supplements, and changes in plasma volume during pregnancy (Klebanoff *et al.,* 1991). However, Hb concentration lower than 11 gams per decilitre (g/dL) at sea level is regarded as pregnancy related anaemia. Anaemia during pregnancy is further classified into mild (10.0 – 10.9 g/dl), moderate (7.0 – 9.9 g/dl) and severe (less than 7 g/dl) (WHO, 2011).

Globally, 43 % of pregnant women are anaemic (Burden *et al.,* 2023). Studies in Ghana have reported varied prevalence. A cross-sectional survey involving 378 pregnant women in the West Gonja District of Ghana recorded 56% prevalence of anaemia for women at their first antenatal visit (Hb at booking) (Tibambuya *et al.,* 2019). It predicts pre-pregnancy maternal serum iron status, especially if the measure was taken earlier in pregnancy, at the time the blood volumes have not been greatly influenced by the changes in their body physiology resulting from pregnancy. Wemakor and colleagues observed anaemia prevalence of 50.8 % from a cross-sectional study comprising 400 pregnant women (Wemakor, 2019) in a tertiary referral hospital

in Northern Ghana. A 40.8 % prevalence was also reported by Anlaakuu & Anto in Sunyani, Ghana (Anlaakuu & Anto, 2017).

Research studies find association of maternal pregnancy haemoglobin and adverse pregnancy outcomes like SPTB to take a U-shaped form (Chehaibi *et al.,* 2016; Dewey & Oaks, 2017). It is a modifiable risk factor that is associated with several adverse pregnancy outcomes including SPTB. Maternal anaemia reduces the oxygen-carrying capacity of the blood, resulting in insufficient oxygen and nutrients reaching the placenta and, subsequently, the foetus. It also reduces the immunity of the pregnant woman against infection, a modifiable risk factor for SPTB (Lye *et al.,* 2021).

A cross sectional study in Brazil involving 4764 women found anaemia to be associated with the risk of preterm birth (19.7% vrs 7.9 %, $p < 0.001$) (Figueira *et al.,* 2021). A Finnish study of 215 pregnancies found iron deficiency anaemia to be associated with preterm birth (10.2% vs. 6.1%, $p = 0.009$) (Kemppinen *et al.,* 2021). A study in Saudi Arabia also recorded increase prevalence of preterm birth from 9% among women with moderate anaemia to 15% among women with severe anaemia (< 7 g/dl) (Aboushamat & Nanu, 2016). In a prospective, multinational study conducted in Brazil, Kenya, Pakistan, South Africa, and the UK, Ohuma and colleagues found serum haemoglobin concentration 7.0g/dl to be associated with increased risk SPTB by over 2-folds, RR = 2·04, 95% CI: [1.20 – 3.48] and 16.5 g/dl to be associated with increased risk of preterm by over 2-folds, RR = 2.06, 95% CI: [1.41 – 3.02] (Ohuma *et al.,* 2023).

Hb levels beyond 16.5 g/dl for women is regarded as polycythaemia. The mechanisms through which high iron status during pregnancy adversely affects birth outcomes includes: oxidative stress, increased blood viscosity, and impaired systemic response to inflammation and infection (Dewey & Oaks, 2017). Excess iron is stored in ferritin in a safe and inert form. However,

excessive accumulation of iron in the body can overwhelm ferritin's capacity to sequester iron. This excess iron becomes free or labile iron which can participate in Fenton and Haber-Weiss reactions represented as follows:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$

In this reaction, iron (Fe^{2+}) reacts with hydrogen peroxide (H_2O_2) to produce hydroxyl radicals (•OH), which are highly reactive and can initiate oxidative damage to cellular components. The Hydroxyl radicals can cause oxidative stress which can damage lipids, proteins, and DNA or stimulate the release of pro-inflammatory cytokines which can lead to SPTB (Strohmaier *et al.,* 2019)

Increased blood viscosity results in reduced oxygen delivery to tissues like the placental tissues which also disrupt oxygen and nutrient supply to the foetus (Woo *et al.,* 2023). A study by Saigo and colleagues found a positive correlation between derivatives of reactive oxygen species and serum ferritin levels ($p = 0.0117$) (Saigo *et al.,* 2011). Several works have confirmed a positive association between increased haemoglobin and oxidative stress, risk factor associated with SPTB (Chehaibi *et al.,* 2016; W. Liu *et al.,* 2011; Plewes *et al.,* 2017).

2.16. SPTB and Neonatal characteristics

Preterm birth results in the truncation of intrauterine duration that the foetus needs to mature before exposure to the environment outside the womb (WHO, 2018). The unmatured state predisposes neonates to many medical complications such as: respiratory distress, unstable body temperature, jaundice, hypoglycaemia, apnoea, seizures, and feeding difficulties, compared to their term counterparts (Eichenwald *et al.*, 2016; Tana *et al*., 2023; Twanow, 2022; Wang 2023). These developmental deficits are also influenced by factors including: maternal nutrition (Habtu

2022; Karim *et al.*, 2023); maternal health condition such as diabetes and hypertension that may result in intrauterine growth retardation (IUGR) (Bartels *et al*., 2020). Neonatal development is also influenced by: placental function (Gasiorowska *et al*., 2019); and environmental factors such as maternal medications, especially, repeated courses of antenatal corticosteroids (Norberg *et al.,* 2011; Sacchi *et al.,* 2021), maternal exposure to toxins, smoking, socioeconomic status (Bramsved *et al.*, 2023; Delcroix *et al.*, 2023) and infection (Wright *et al*., 2023). The truncation of the gestational age also results in deficits evident in the neonatal anthropometries including: head circumference, length and birth weight, and the 1st and 5th minute Apgar scores.

2.16.1. Birth weight

Birth weight is a determinant of neonatal outcome and long-term health. A lower birth weight below the 10th percentile for a gestational age is described as small for gestational age (SGA). Similarly, a neonate is large for gestational age (LGA) if the birth weight is higher 90th percentile for a gestational age. They are determined by comparing the neonate's weight to standard growth charts or percentiles for the specific gestational age (Elmrayed *et al.*, 2023; Lin *et al.*, 2024; Liu *et al.*, 2023).

Both SGA and LGA is associated with birth complications and an elevated risk of cardiovascular disease in their later years by 38% (Lu *et al.,* 2023). Birth weight of less than 2500 grams (2.5 kg) or 5.5 pounds is regarded as low birth weight (WHO, 2024). It is much prevalent in Sub-Saharan Africa. Burkina Faso, Senegal, Uganda, Malawi and Ghana have the prevalence of 13.4%, 15.7% 10%, 12.1% and 10.2%, respectively (Banchani & Tenkorang, 2020; He *et al*., 2018). The truncation of the gestational age of preterm birth children predisposes them to low

birth weight (Algameel *et al*., 2020; Shi *et al*., 2020). A study in Benin found the mean birth weight for both male and female term neonates to be 2985 g.

2.16.2. Head circumference (HC)

It is the measure of the occipitofrontal circumference of the neonate's skull with a flexible, non-stretchable measuring tape or any specialised infant HC measuring tape. It is a rapid, easy and non-invasive method of determining neonatal brain size and serves as a clinical tool for identifying brain anomalies (Selvanathan, 2022). About half of the human brain consists of the cerebral cortex, with most neurons generated by 21 weeks of gestation. A diminished head circumference is likely linked to a relatively smaller cerebral cortex with fewer neurons, making it a proxy marker for neural growth (Woods, 2004).

Comparatively, preterm birth children have smaller HC than the term neonates partly because of the disparity in their gestational periods. However not all preterm children have small head circumference per gestational age (Algameel *et al*., 2020). If a growth catch up is not achieved, it could lead to consequences including neurodevelopmental delays and impairments in areas like gross motor skills, visual-motor coordination, language development, and an increased risk of epilepsy (Center for Disease Control and Prevention, 2016; Nawathe 2018).

Pam and colleagues reviewed the birth records of neonates at a teaching hospital in Nigeria and found the mean head circumference to be 34.4 ± 2.1 cm (male = 34.6 ± 2.16, female = 34.1 ± 2.02 cm)(Pam *et al*., 2019).

2.16.3. Birth length

It is also known as crown-to-heel length or crown-to-rump length. It refers to the measurement of a neonate from the top of the head (crown) to the bottom of the feet (heel) or from the top of the head to the buttocks (rump). It is an anthropometric measurement frequently taken at birth to assess the neonate's size and proportionality. It is associated with adult height and fat-fee mass. It is typically recorded in centimetres or inches (Jamshed *et al.*, 2020). Reduced length is a sign of chronic deprivation of the neonates the nourishment to develop (Christian, 2022; Krebs *et al.*, 2022; Negrato & Gomes, 2013).

A South Korean study of 843 term neonates has set birth length cut-off value for SGA (<10th percentile) at 48 cm for both male and female neonates, and the ≤3rd percentile cut-off at 47 cm for males and 46 cm for females born from 38 to 41 weeks. A study in Benin found a mean birth length of 48.7 cm for both male and female neonates (Padonou *et al.*, 2019). A study in Nigeria found the mean length of female neonates to be (49.0± 0.2) and the male neonates to be (48.9± 0.2) even though not statistically different ($p > 0.05$)(Taiwo *et al*., 2021).

2.16.4. First and fifth minute Apgar scores.

The Apgar score developed in 1952 by an obstetrical anaesthesiologist, Dr. Virginia Apgar (1909 - 1974) has been used globally to assess neonatal [**A**ppearance (Skin colour), **P**ulse (heart rate), **G**rimace (reflexes), **A**ctivity (muscle tone) and **R**espiration (breathing rate) of neonates in the first and fifth minutes of life. It is a standardised critical technique needed to appraise neonatal resuscitation and survival. Serendipitously, the physician's name (Apgar) depicts the constituents of the score (Rozycki & Yitayew, 2023). Each of the five parts the Apgar score is assigned a value 0, 1, or 2 which are then added to produce a score recorded at 1 and 5 minutes after birth.

A score of 7 to 10 is considered good to excellent, 4 to 6 is low or moderately abnormal, and a score of 0 to 3 is deemed very low to low. An infant with a score of < 7, need to be resuscitated and assessed at 5-minute intervals up to 20 minutes (Simon *et al.,* 2024).

Reduce Apgar score has been associated with increased risks of mortality and neurodevelopment (Shah *et al.,* 2022). It is also associated with 2-fold increased risk of cardiovascular diseases in early adulthood (Razaz *et al.,* 2023) and 76% increased risk of attention deficit hyperactive disorder (ADHD) in children (Bala *et al.,* 2023). Based on the health of the neonate, each of the constituents (**A**ppearance, **P**ulse, **G**rimace, **A**ctivity and **R**espiration) is rated a with a score of 0, 1, or 2. For liveborn neonates, a total score of 1 to 10 is assigned. The higher the score, the better the neonate's health after birth. A score of 7, 8 and 9 is a sign that the neonate is in good health. Deceasing Apgar score is associated with increased neonatal mortality and morbidity (Rozycki & Yitayew, 2023). A score of 0 to 3 is regarded as very low score (Dassah *et al.,* 2014). About 6.49% and 1.9% of live birth with very low Apgar scores at 1 and 5 minutes, respectively was recorded in a retrospective review of vaginal deliveries at a tertiary hospital in Ghana (Dassah et al., 2014). In a Slovenian study involving 11,924 children, Apgar score less than 7 was found to be associated with over 8-fold increased and almost 5-fold increased risk of cerebral palsy among children with Apgar scores 0 – 4 and 5 – 6, respectively.

2.17. Placental Histopathology and SPTB

The placenta serves as the primary organ during pregnancy, controlling vital metabolic, respiratory and endocrine processes. It also functions as a safeguard, protecting the foetus from infections and environmental influences throughout gestation. This pivotal role of the placenta significantly shapes foetal development and the potential for foetal mortality or neonatal health

challenges. Compromised placental task could activate a range of pregnancy complications, including SPTB (Matoba *et al.,* 2021).

SPTB attributed to placental dysfunction can result from factors such as intrauterine infection or inflammation, haemorrhaging and reduced foetal-placental blood circulation leading to hypoxic-ischemic events (reduced oxygen circulation). Analysis of the placenta through both macroscopic (gross) and microscopic (histologic) examinations has contributed to elucidate these mechanisms underlying SPTB. A histopathological assessment of the placenta has identified some histologic lesions related to altered structural and inflammatory responses (Ericksen *et al.,* 2020).

An example of placental inflammation is chorioamnionitis. It is the inflammation of the foetal membranes (chorion and amnion) and the placenta. This inflammation is often caused by infection. Bacterial infections are common and raise the risk of preterm delivery. It is a major contributor to all forms of preterm births, with histological chorioamnionitis increasing with decreasing gestational weeks. In a study of 43 mothers with preterm deliveries less than 35 weeks, the frequency of histological chorioamnionitis increased from 47.3% among deliveries <32 weeks to 83.3% for deliveries less than 30 gestation weeks (Erdemir *et al.,* 2013). Increased placental pathologies is exacerbated by lifestyles such as smoking and alcoholism (Sprong *et al.,* 2023).

Surprisingly, even in full-term elective caesarean sections, bacterial invasion and inflammation could be observed in over two-thirds of the placental biopsies (Steel *et al.,* 2005). Recently, Sprong and colleagues in a study found an association between acute chorioamnionitis and term births in a high-risk population in South Africa ($p < 0.002$) (Sprong *et al.,* 2023). Similarly, Ericksen and colleagues found chorioamnionitis to be much more related with term birth than SPTB (50% vs. 17.8%, $p < 0.001$) and lowered the risk SPTB (OR = 0.33; 95% CI: [0.13 –

0.79]; $p < 0.05$) (Ericksen *et al.,* 2020). Studies have found placental bacteriologic assessment to be unreliable for predicting chorioamnionitis and to a large extent preterm birth, based on outcomes from postpartum placental bacteriologic cultures. This suggests that the placenta used for such analysis becomes contaminated by the vaginal microflora during delivery and could give false positive outcome on the association between infection and inflammation of the placenta (Berezowsky *et al.,* 2022). It is shown histologically as neutrophilic infiltration (especially polymorphonuclear leukocytes) in the chorion and amnion (McManus & Mitchell, 2014) .

The pathogenesis of SPTB without infection may entail the activation of pathways associated with normal onset of labour via elements of the maternal and foetal hypothalamic–pituitary–adrenal axis (Faye-Petersen, 2008). It results from trauma which disrupts the integrity of the placental tissues and release cellular contents, including damage-associated molecular patterns (DAMPs). This activates immune cells to release pro-inflammatory signalling molecules and prostaglandins to increase blood vessels permeability and result in the invasion of neutrophils and macrophages (Romero et al., 2011). Additionally, oxidative-stress-induced-damage as well as early senescence in the placenta is another predisposition to SPTBs (Menon *et al.,* 2020; Romero *et al.,* 2011)

Another aetiology is uteroplacental malperfusion resulting in decreased blood flow between the uterus and the placenta (Sprong *et al.,* 2023). It is associated with over 4 out of 10 spontaneous preterm cases (Jaiman *et al.,* 2021). This can be exhibited in the placental tissue as increased syncytiotrophoblastic knots or syncytial knots. These represent clusters of syncytial cell nuclei located on the surface of terminal villi. The syncytiotrophoblasts are specialized cells with multiple nuclei within a single continuous cytoplasmic mass that form a protective barrier

between maternal and foetal blood, transport nutrients from the maternal circulation to the foetal circulation and secrete hormones, including human chorionic gonadotropin (hCG) and human placental lactogen (hPL), vital for pregnancy (Loukeris *et al.,* 2010). The syncytial knots however increase in number as gestational age progresses, offering a means to assess the maturity of villous structures (Apel-Sarid *et al.*, 2010).

Another lesion observed in the placentas of SPTBs is advanced maturation of the chorionic villi relative to the gestational age, also known as placental accelerated villous maturation (PAVM). It has features such as increased branching. The villi near the maternal surface (decidua basalis) becomes elongated and small for the gestational age. Jaiman and colleagues, 2021 reported a 32% prevalence in a study involving 333 placental tissues study of singleton pregnant women with SPTB (Jaiman *et al.,* 2021). In another study in the US, Jaiman and colleagues found accelerated villous maturation to be present in 9.5% in 95 placental tissues from SPTB women but none in 519 controls (Jaiman *et al.,* 2022). Accelerated villous maturation was also found to be associated with almost 18-fold increase in SPTB in a study in a South African study (Brink *et al.,* 2022).

Distal villous hypoplasia is another form of placental malperfusion characterised by reduced branching of the terminal portions of the placental villi, especially, the terminal villous tree. These villi also appear thin and elongated and usually wide intervillous spaces (Fitzgerald & Keating, 2018; Nijman et al., 2016). The condition results in reduced surface area for effective maternal-foetal exchange. Maternal health conditions such as undernutrition in situations like folate and iron deficiencies (Chilukuri *et al.,* 2022; Rakoczy & Watson, 2023; Toblli *et al.,* 2012) as well as conditions such as hypertension, diabetes and autoimmune disorders affect placental development and could result in distal villous hypoplasia (Brink *et al.,* 2022; Kulkarni *et al.,*

2021; Susana *et al.,* 2023). Its prevalence in the SPTB placental tissue is reported to be 9% (Brink *et al.,* 2022) 1.7% (Nijman *et al.,* 2016) and 1.4% (Jaiman *et al.,* 2021). It was much observed in indicated preterm births compared with SPTB (Brink *et al.,* 2022). Jaiman and colleagues reported a significant association between distal villous hypoplasia and SPTB (prevalence ratio = 12.9, $p < 0.01$) (Jaiman *et al.,* 2021).

There could also be other structural changes of the placental villi in the form of infarctions (areas of tissue death), fibrinoid necrosis (degeneration of connective tissue) and increased deposition of fibrin around the villi (increased perivillous fibrin deposition), also indicative of malperfusion (Catov *et al.,* 2017; Mestan *et al.,* 2014; Zaidi *et al.,* 2013). Infarction in the placental tissue could be macroscopic, appearing as white, pale-yellow to reddish-brown tissue depending on the stage of infarction and the presence of associated haemorrhage observable on the placental surface or a section though the placenta. The microscopic infarction typically presents itself as pale or eosinophilic regions with loss of cellular detail and architectural integrity. Cellular debris in the form of karyorrhexis (nuclear fragmentation) and pyknosis (shrunken nuclei) could be observed within necrotic regions. Sometimes, the necrotic tissue may be infiltrated by neutrophils and macrophages as part of an inflammatory response. In a case-control study of 944 term placenta and 438 SPTB placentas, Jaiman and colleagues did not find any significant association between villous infarct and SPTB (Jaiman *et al.,* 2021). Brink and colleagues observed microscopic infarction in 14% (20/141), 8% (72/876) and 31% (26/84) placental samples from SPTB, spontaneous term and indicated preterm birth placentas, respectively. Nijman and colleagues also observed microscopic infarction in 3.3% (4/121) and 18.9% (21/111) of placental samples from SPTB and indicated preterm birth placentas, respectively.

Fibrinoid necrosis appears as fibrin-rich structures in the form of islands within the placental tissue. They are characterised by a glassy or waxy, homogeneously pink eosinophilic material situated between the placental villi or in the intervillous space and associated with impaired placental perfusion (Parks, 2015). They occur due to several factors including maternal hypertensive disorders, systemic lupus erythematosus and antiphospholipid antibody syndrome including idiopathic causes as well. However, a common characteristic feature is a suboptimal remodelling of maternal spiral arteries in the placenta. This result in an acute atherosis in the form of fibrinoid necrosis (Redline *et al.,* 2004). Their presence impairs placental perfusion and is associated with adverse pregnancy outcomes such as preterm birth, intrauterine growth retardation (IUGR), and stillbirth. According to Brink and colleagues, much of fibrinoid island appears to be associated with indicated preterm birth than SPTBs, especially, resulting from hypertensive disorders (Kulkarni *et al.,* 2021). Brink and colleagues reported a prevalence of 18% (26/141) from SPTB placental tissues compared with 33% (28/84) from indicated preterm birth placental tissues (Brink *et al.,* 2022). Jaiman and colleagues also found the prevalence of fibrinoid necrosis to be 12.3% (54/438) to be significantly associated with SPTB compared with 6.4% (60/944) for the controls (term births placenta) [$p < 0.001$, (prevalence ratio = 1.9)] (Jaiman *et al.,* 2021).

Placental malperfusion can also present itself in the form of increased perivillous fibrin deposition (Faye-Petersen & Ernst, 2013). This results from the build-up of fibrinoid tissue closely adjacent to the chorionic villi and obstruct utero-placental perfusion. They are observed in placental tissues with pathologies associated with vascular injury, inflammation or impaired placental perfusion (Kulkarni *et al.,* 2021). Brink and colleagues identified the prevalence of increased perivillous fibrin to be 30% (42/141), 50% (439/876) and 55% (46/84) in the placental

tissues obtained from women with SPTB, spontaneous term birth and Indicted preterm birth, respectively.

Villitis of unknown aetiology (VUE) is used to describe inflammation of the placental villi without any recognisable cause. The mechanism underlying the condition is not properly understood. However, immune dysregulation, and maternal conditions such as autoimmune disorders such as alloimmunity where the maternal immune system mounts an immune response against the growing foetus or the vice versa can also be exhibited in the placenta in the form of VUE. Chronic inflammatory conditions have also been associated with it (Dubruc *et al.,* 2016). According to Redline 2007, maternal T lymphocytes mainly CD8-positive gain inappropriate entry to the villous stroma.

Villitis of unknown aetiology (VUE) is documented to manifest in as many as 15% of placentas at full term (Redline, 2007). In a study of 102 placentas, (34 with VUE and 68 without VUE) there was no statistically significant difference between the number of SPTB with VUE (39.1%) and those without VUE (45.6%), $p < 0.62$. However, patients with VUE had increased rate of previous SPTB compared with patients without VUE (26.9% vs. 7.7%, $p < 0.04$) (Iskender *et al.,* 2017). Nijman and colleagues found VUE to be 9.1% in SPTB placental samples and 17.1% in indicated preterm birth placental samples (Nijman *et al.,* 2016).

2.18. Genetic Contribution to SPTB

One of the strongest risk factors for SPTB is a history of preterm delivery. However, SPTB population is highly heterogenous, making the investigation into the genetics of SPTB quite challenging (Zhang *et al.,* 2015). These genetic factors interact with numerous environmental factors to trigger common signalling pathways resulting in SPTB (Nadeem *et al.,* 2019).

Observations including recurrent preterm births (Smid, Jong, *et al.,* 2017; J. Yang *et al.,* 2016) suggest the involvement of the maternal genome to SPTB occurrence. The risk of SPTB is estimated to be 2.5-fold to 4-fold (Hammond *et al.,* 2013; Mercer *et al.,* 1999; Smid, Jong, *et al.,* 2017; J. Yang *et al.,* 2016) among women with recurrent SPTB compared with those with no prior preterm delivery. Outcomes of epidemiological studies suggests that recurrent preterm births are increased by over 2-fold among women who themselves were born preterm (Smid, Jong, *et al.,* 2017; J. Yang *et al.,* 2016).

The risk increases to over 10-fold as the gestational age of preterm delivery reduces to < 28 weeks (Mercer *et al.,* 1999). Additionally, Bhattacharya and colleagues (2010) observed from 13,845 pregnancies that daughters born to mothers who were themselves born SPTB had 50% increased odds of delivering preterm babies. Nulliparous women who had been born spontaneously preterm had 60% increased odds of delivering preterm babies. Estimates from twin studies indicate that maternal genetic variants account for approximately 27% to 36% of spontaneous preterm birth incidence (Kistka *et al.,* 2008; Treloar *et al.,* 1999; York *et al.,* 2013). Twin studies have observed heritability estimates due to maternal contribution ranging from 15% (Lunde *et al.,* 2007; W. Wu *et al.,* 2015) to 40% (Kistka *et al.,* 2008; Plunkett *et al.,* 2009). Concerning racial differences in preterm birth occurrence, several evidences adjusted for maternal environmental and socioeconomic status have found women of African descent to be at 2 to 3-fold increased risk to SPTB (Martin, Hamilton, & Osterman, 2017; Smid, Lee, *et al.,* 2017), and over 5-fold at risk of recurrent SPTB (Kistka *et al.,* 2007) compared to Caucasians.

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2.18.1. Genetics of Preterm Birth study – methods employed

A range of techniques, including large intergenerational studies (Smid, *et al.,* 2017), candidate gene investigations (Sheikh *et al.,* 2016; J. Song *et al.,* 2017), genome-wide association studies (GWAS) (Huusko *et al.,* 2018) have also been utilized. The research extends to exploring epigenetic modifications (Park *et al.,* 2020), transcriptomic alterations (Gupta *et al.,* 2022), proteomic (Khanam *et al.,* 2019) and metabolomic profiles (Romero *et al.,* 2010) in preterm birth cases and controls.

A larger chunk of research into the genetics of preterm birth has focused on the candidate gene approach. It has focused on polymorphisms related to tissue remodelling (Pereza *et al.,* 2014), inflammatory/immunological (Song et *al.,* 2017), metabolic/biosynthetic (Christiaens *et al.,* 2015), and haematological/vascular/endothelial pathways (Mead *et al.,* 2023). Frey *et al.* (2016) studied 1536 SNPs among 77 African-American women with SPB and 756 controls and found that tag SNPs related to genes for *PROTEIN KINASE C—α* (*PRKCA*) especially, *FELINE MCDONOUGH SARCOMA* (*FMS*)*-RELATED TYROSINE KINASE 1* (*FLT1*), *MATRIX METALLOPROTEINASE-2* (*MMP2*), *TISSUE INHIBITOR OF MATRIX METALLOPROTEINASE-2* (*TIMP2*), *INTERLEUKIN* 16 (*IL16*), *MATRIX METALLOPROTEINASE-1* (*MMP1*) and *LEUKAEMIA INHIBITORY FACTOR RECEPTOR ANTISENSE RNA 1* (*LIFR-AS1*) genes critical to inflammation, extracellular remodelling, and cell signalling were associated with increased risk of SPTB (Frey *et al.,* 2016). A couple of studies mostly concentrated on women of European origin have harnessed GWAS (Zhang *et al.,* 2018).

2.18.1.1. GWAS in preterm birth research

Over the past decade, many Genome-Wide Association Studies (GWAS) have been conducted on preterm birth and gestational duration. However, generally, six studies, where four studies (Gupta *et al.,* 2022; Huusko *et al.,* 2018; Solé-Navais *et al.,* 2023; G. Zhang *et al.,* 2017) used maternal samples and two studies (Liu *et al.,* 2019; Rappoport *et al.,* 2018) used infant samples have produced genome-wide significant results (Mead *et al.,* 2023).

Considering the four maternal studies, a recent extensive maternal genome-wide meta-analysis utilized data from 18 cohorts, encompassing over 190,000 European maternal samples for gestational duration and over 270,000 (18,797 SPTBs and 260,245 term birth) samples for SPTB. This analysis identified genetic variants at 22 loci linked to gestational duration and 6 loci linked to SPTB, all reaching genome-wide significance ($p < 5x10^{-08}$). Despite some overlap between the two phenotypes (gestational duration and SPTB), the genetic correlation between them was moderate (rg = -0.62, 95% CI: [-0.72, to -0.51]), suggesting differing genetic effects between gestational duration and SPTB. Maternal genetics exhibited a larger influence on preterm birth (PTB) compared to foetal genetics, although effect sizes were generally small. However, attempts to predict SPTB outcomes using the identified variants showed poor predictive performance (area under the receiver operator characteristics) (AUROC = 0.61, OR = 0.69, 95% CI [0.56 – 0.85]) (Mead *et al.,* 2023; Solé-Navais *et al.*, 2023)

Zhang *et al.* (2017) performed a GWAS of over 40,000 indigenous European women (3331 preterm, and 40,236 term births), and replicated with samples from 8643 women from three Nordic data sets. They found four loci, namely: *EARLY B-CELL FACTOR 1* (*EBF1*), *EUKARYOTIC ELONGATION FACTOR, SELENOCYSTEINE-TRNA SPECIFIC* (*EEFSEC*), *ANGIOTENSIN II RECEPTOR TYPE 2* (*AGTR2*), and *WNT FAMILY MEMBER 4* (*WNT4*) to be

significantly associated ($p < 5.0\times10^{-8}$) with gestational duration, while *ADENYLYL CYCLASE TYPE 5 ADCY5* and *RAT SARCOMA (RAS)-RELATED PROTEIN 2C RAP2C* had suggestive significant association *($p < 1.0\times10^{-6}$)* with gestational duration. Variants in *EBF1*, *EEFSEC*, and *AGTR2* showed significant association with preterm birth (Zhang *et al.,* 2018). Huusko *et al.* in 2018 conducted a whole exome sequencing to uncover rare, potentially harmful genetic variants contributing to recurrent SPTB in Finnish and Danish populations. Discovery analyses of 17 Finnish mothers with SPTB history and 93 Danish sister pairs revealed shared variants in genes, particularly in the glucocorticoid receptor signalling pathway to be most significant ($p < 1.7\times10^{-8}$). *HEAT SHOCK PROTEIN FAMILY A MEMBER 1-LIKE* (*HSPA1L*) gene had damaging alleles, notably rs34620296, with higher frequency in cases than controls (0.0025 vs. 0.0010, p = 0.002) (Huusko *et al.,* 2018). Gupta *et al.* (2022) published a GWAS exploring maternal genetics and early preterm birth sub-phenotypes. Studying 310 Caucasian women, SNP rs14675645 (*ASTN1*) showed genome-wide significant association with SPTB (Gupta *et al.,* 2022).

2.18.2. The -656(C >T) *SERPINH1* and SPTB

The *SERPINH1* gene plays a critical role in collagen metabolism. Rocnik *et al*.'s research found varied interindividual *SERPINH1* gene expression and a polymorphism [(-656) C > T] in the gene's promoter among African Americans which significantly decreased promoter activity. This provided the rationale to research the role of the *SERPINH1* gene in SPTB (Rocnik *et al.,* 2002). Consequently, and based on the increased prevalence of preterm birth among African Americans compared to Caucasians, Wang *et al*. 2006 investigated the contribution of the (-656) C >T SNP to preterm premature rupture of membranes (PPROM) among African Americans. Initially, they assessed the -656 T allele distribution from 919 peoples from well characterised groups

ethnic/racial backgrounds and found an increased T allele frequency (12.4% vs. 4.1%, $p <$ 0.024) in the African Americans compared with the European Americans. Among the African population, Wang and colleagues found the T allele frequency to be between 0.11 and 0.25 (**Table 2.1**).

Wang and colleagues then conducted a promoter function studies to compare the activities of the T and C alleles using three different human cell types (amnion fibroblasts cells, dermal fibroblasts and uterine smooth muscle cells). The human amnion fibroblasts cells demonstrated significant reduction in promoter activity for the -656 T compared to the major 656 C allele ($p <$ 0.05).

Afterwards, a case-control study as part of Wang *et al.*'s work using cord blood from 244 cases (pregnancies complicated by PPROM) and 358 controls (neonates born at term) demonstrated a significantly increased -656 T allele frequency (11.5% in cases and 4.05% in controls) among the African-American neonates compared with controls (OR = 3.22,95% CI: [1.50 – 7.22]; $p <$ 0.0009). A test for trend of allele dose effect for the -656 T allele was statistically significant ($p <$ 0.002) for PPROM. The risk of the heterozygotes for the T allele to PPROM was higher than homozygotes for the common C allele (OR = 2.68, 95% CI: [1.45 – 4.4.95]). The homozygotes for the T allele even had a higher risk compared with heterozygotes for the T allele (OR = 2.68, 95% CI [1.49 – 4.95]). Adjusting for admixture, association between the -656 T allele and PPROM was still statistically significant (OR = 3.14, 95% CI: [1.53 – 4.47], $p <$ 0. 002).

In a follow-up case-control study using a different cohort of 184 controls and 92 cases, neonates with the -656 T allele were significantly associated with PPROM (-656 T allele frequency: cases, 11.41%; controls, 5.16%; $p <$ 0.0076; OR = 2.37, 95% [CI: 1.17 – 4.79]. Merging the two case-control studies (cases = 244; controls = 358) a significant association between the -656 T allele

and PPROM was determined (-656 T allele frequency: cases, 11.48%; control, 4.47%; $p < 0.0000045$; OR = 2.77; 95% CI: [1.73 – 4.95]) (Wang et al., 2006).

Subsequently, Wang and colleagues detected a 12-base-pair deletion in the 5'-flanking of the *SERPINH1* gene in linkage disequilibrium and with the [(-656) C > T]. This deletion was found to increase the promoter activity of the *SERPINH1* gene in fibroblast cells with the *SERPINH1*-656 T allele. However, the frequency of the 12-base-pair deletion was much lower compared with the *SERPINH1*-656 T allele, especially, in the African population except the Temne ethnic group in Sierra Leone (**Table 2.1**).

Table 2.1. Ethnic distribution of the *SERPINH1* promoter -656 "T allele" and 5'-flanking 12-bp deletion frequencies

Country (ethnic group)	Sample size	*SERPINH1* -656 "T" allele frequency	*SERPINH1* 5'-flanking 12-base-pair deletion frequency
Caucasians	148	0.041	0.014
Bolivia	92	0.046	0.04
Guatemala (Mayan)	40	0.075	0.04
Mexico	144	0.056	0.05
South Asia	140	0.046	0.04
China	43	0.083	0.06
Nigeria	76	0.11	0.01
Sierra Leone (Creole)	37	0.24	0.00
Sierra Leone (Fula)	7	0.21	0.00
Sierra Leone (Limba)	23	0.24	0.00
Sierra Leone (Loko)	9	0.17	0.00
Sierra Leone (Mandigo)	8	0.125	0.00
Sierra Leone (Mende)	93	0.13	0.06
Sierra Leone (Temne)	59	0.25	0.22

Table 2.1 *was adopted from "A 12-bp deletion in the 5'-flanking region of the SERPINH1 gene affects promoter activity and protects against preterm premature rupture of membranes in African Americans" (Wang et al., 2008). The base-pair deletion frequency varied from 0.00 in the Creole, Fula, Limba, Loko, and Mandigo ethnic groups to 0.22 Temne in the African population. Outside Africa, the 12-bp deletion frequency varied from 0.014 among the Caucasians to 0.06 in the Chinese. On the other hand the -656 T allele frequency range between 0.11 and 0.25 in the African population compared with a range between 0.041 to 0.083 in population outside Africa.*

2.18.2.1. *SERPINH1* gene and collagen metabolism

The *SERPINH1* gene, approximately 10782 bases in length, positioned at Genome Reference Consortium human genome build 38 (GRCh38) chromosome 11:7562056 – 75572783 codes for a serine proteinase inhibitor known as HSP47 protein. The HSP47 is a glycoprotein functioning

as an endoplasmic reticulum (ER) molecular chaperone. HSP47 has 31% sequence homology with serine proteases and 10-30% with other serpin family members (Hirayoshi *et al.,* 1991). The *HSP47* gene features a conserved heat shock element (HSE) around -180 base pairs from the transcription start site (**Fig. 2.3**), responsible for its heat inducibility. Heat shock and other stresses like arsenite exposure and unfolded protein accumulation in the cytoplasm activate the heat shock factor 1 (HSF1), which binds and activates *HSP47* mRNA transcription. The HSP47 is distinct as the sole ER-localized heat-inducible protein responding to unfolded protein accumulation (Nagata, 2003). **Figure 2.3** also shows the -656 (C > T).

Specificity protein-1 (Sp-1), a member of a sp family proteins characterizes highly conserved DNA-binding domain consisting of three zinc finger motifs has a binding site situated 210 bases from the transcription initiation site. This binding site is a principal initiation site for basal transcription. Tissue-specific expression of *HSP47* is activated by two *cis*-acting elements, namely: BS5-B and EP7-D, located in the first and second introns, respectively. The BS5-B is a binding site for two members of the specificity protein (sp) family of proteins; Sp2/Sp3, and zinc finger protein 9 (Zf9), a Kruppel-like factor. The binding factor(s) for EP7-D however, has/have not been identified (Ishida & Nagata, 2011; Yasuda *et al.,* 2002).

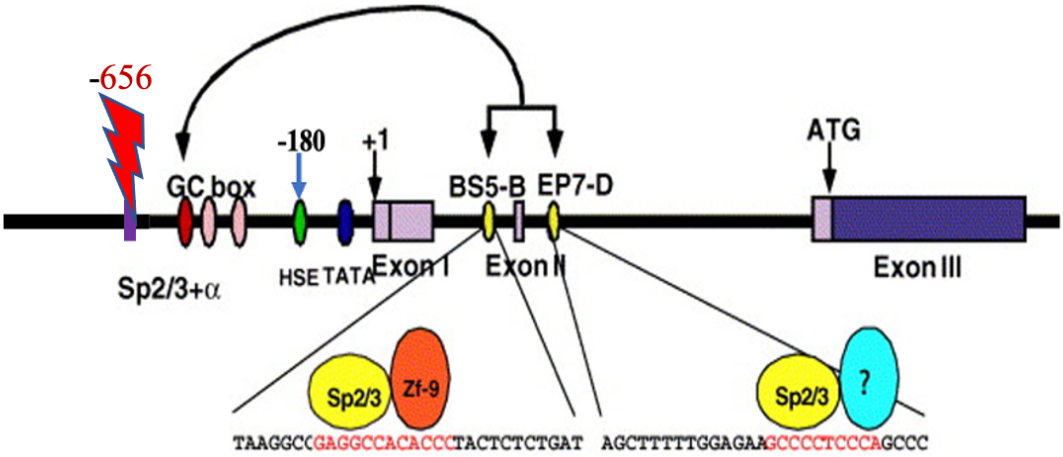


Figure 2.3: The promoter region of the SERPINH1 gene. *The GC box and two cis-acting elements BS5-B and EP7-D in the first and second intron respectively are responsible for expression of hsp47. The Sp2/Sp3, and Zf9 are transcription factors that bind to these regions (Nagata, 2003). The -656 (C/T) SNP is located upstream of the GC box, not drawn to scale.*

The chaperone activity of Hsp47 in collagen formation in Hsp47 wild-type (WT) cells and Hsp47 knock-out (KO) cells are demonstrated in **Figure 2.4**. The α-chains of procollagen enters the lumen of the ER for the formation a triple-helical configuration via hydroxylation by prolyl 4-hydroxylase (P4H) and intra-chain and inter-chain disulphide bonds formation by protein disulphide isomerase (PDI). The Triple-helix configuration advances from the C-terminus to the N-terminus to preserve the α-chains in an unfolded state for trimer formation. The procollagen is then transported from the ER to the cell surface through the Golgi apparatus under acidic pH via vesicular transport (Oecal *et al.,* 2016; Saga *et al.,* 1987). At the cell surface, N- and C-propeptide peptidases cleaves the N- and C-propeptides, respectively, producing a triple-helix mature collagen. The mature collagen is hydroxylated and renders them resistant to proteolysis by proteases like pepsin, trypsin and chymotrypsin (Ito & Nagata, 2019). Diverse collagen types share a triple helix structure of α-chains forming homotrimers or heterotrimers. There are 29

vertebrate collagens, labelled by Roman numerals in order of discovery. (Sorushanova *et al.,* 2019).

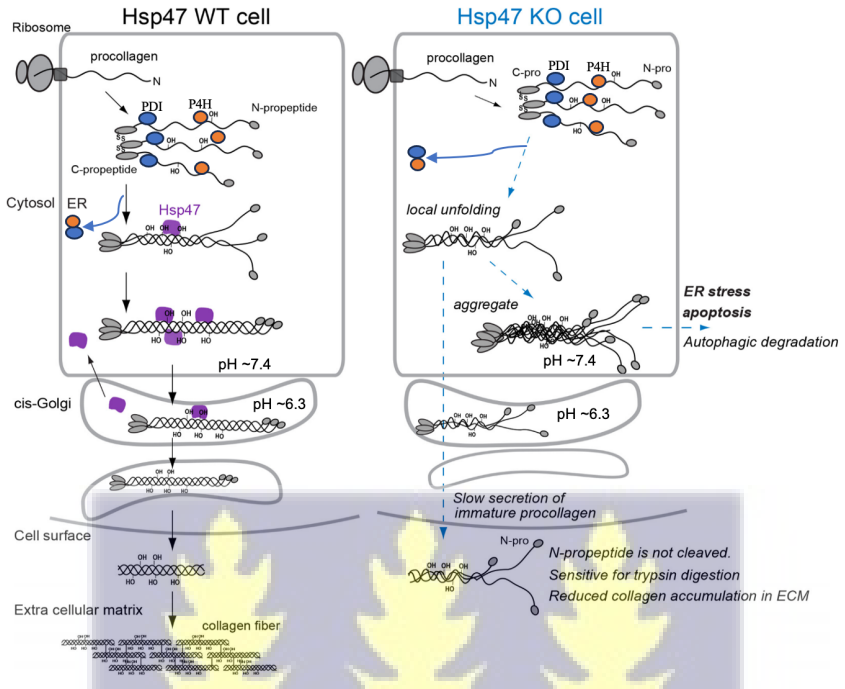


Figure 2.4: Procollagen folding in the ER between Hsp47 WT and Hsp47KO cells. *The newly formed procollagen is transported into the ER and assembles into a trimer with a triple-helical structure. The collagen-specific molecular chaperone, Hsp47 then binds to the triple-helical procollagen to maintain its stability and prevent it from local unfolding and aggregation. The Hsp47 dissociates from procollagen under low pH conditions in the cis-Golgi apparatus. Collagen folding is however compromised and some portions of procollagen are retained in the ER of Hsp47 KO cells (Ito & Nagata, 2019).*

2.18.2.2. Collagen and sustenance of pregnancy

Collagen, the most abundant protein in mammals, constitutes the extracellular matrix (ECM) that provides tissue strength, cell adhesion, and influences cell differentiation and shape (Gelse *et al.,* 2003). The ECM's composition, structure, and function differ across tissues due to distinct protein expression. Pregnancy requires ECM growth and remodelling, with collagen playing a pivotal role in tissue integrity and influencing various physiological processes, including changes in organs like the uterus, cervix, and foetal membrane (Senapati *et al.,* 2018). The cervix,

comprised mainly of connective tissue and limited smooth muscle (< 15%), relies on a collagen-rich ECM for its essential biomechanical properties (Danforth, 1947).

Pregnancy demands a significantly stronger uterus to accommodate foetal growth. Studies show a remarkable eight-fold increase in collagen content in the pregnant human uterus at term compared to the non-pregnant state (Morrione & Seifter, 1962). Additionally, research demonstrates a two-fold increase in type I collagen and five-fold increase in type III collagen in the ECM of pregnant women myometrium at term compared to non-pregnant women (Stewart *et al.,* 1995).

The foetal membrane's ECM, primarily composed of fibrillar collagens I and III cross-linked by types V and VI collagens, along with fibronectin, and laminin maintains its mechanical integrity (Hampson *et al.,* 1997; Kanayama *et al.,* 1985; Malak *et al.,* 1993; Stewart *et al.,* 1995). However, the concentration of collagens 1 and III is reduced in the foetal membrane of PPROM compared with the foetal membrane of term pregnancy (Kanayama *et al.,* 1985).

The balance between formation and breakdown of ECM components influences foetal membrane strength (Wang *et al.,* 2006). Key elements in the amnion's tensile strength are thought to be fibrillar collagens (types I, III, and V), along with other extracellular matrix proteins like type IV and VI collagen, fibronectin, and laminin (Malak & Bell, 1994; Moore *et al.,* 2006). Connective tissue disorders that impact fibrillar collagen formation or ECM protein structure could compromise foetal membrane tensile strength, leading to preterm birth. Collagens are largely resistant to proteolytic degradation and proteolysis can only be mediated by matrix metalloproteinases (*MMPs*) (Shoulders & Raines, 2009).

2.18.3. Matrix Metalloproteinases and pregnancy

The MMPs are calcium and zinc-dependent peptide hydrolases secreted from cells that regulate the ECM via degradation of the ECM molecules (Shi *et al.,* 2020). They have been grouped based on function and structure into collagenases, gelatinases, matrilysins, stromelysins and membrane-type MMPs proteinases depicting their substrates (Murphy, 2016; Tallant *et al.,* 2010).

The host produces different types of MMPs to degrade and remodel tissues. MMP1 (interstitial collagenase) degrades collagens Types I, II and III; MMP8 (neutrophil collagenase) degrades Types I and III; and MMP2 and MMP9 (gelatinase) degrade Type IV and V collagens. In healthy states, precise *MMP* gene expression is governed by transcriptional, post-transcriptional regulation, and diverse factors through signalling pathways. This maintains physiological functions, including tissue remodelling and wound healing (Shiomi *et al.,* 2010). Conversely, elevated MMP levels in disease states cause tissue damage, linked to conditions like atherosclerosis, inflammation, cancer spread, arthritis, and periodontitis. During pregnancy, increased prostaglandin release derived from increased inflammatory cytokine synthesis due to infection, uterine stretch, and increased CRH due to foetal or maternal stress regulates *MMP* expression as shown in **Figure 2.5.**

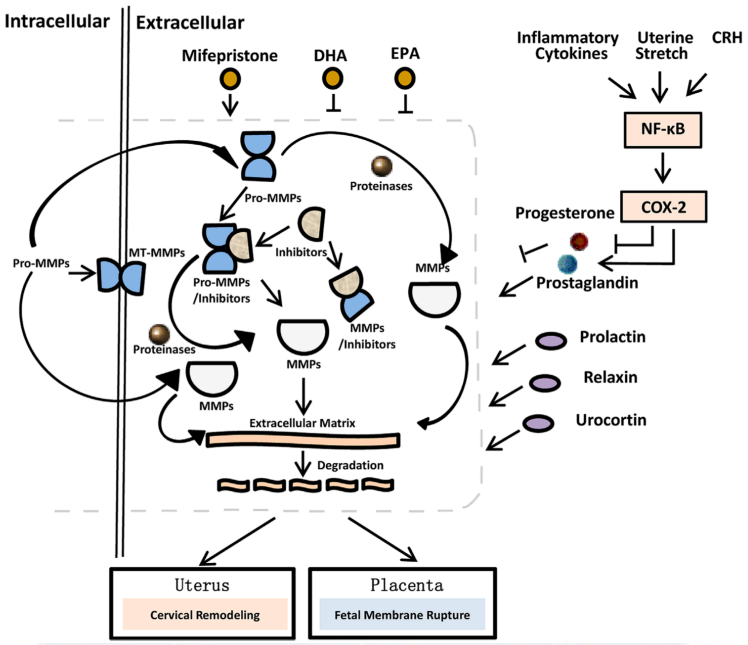


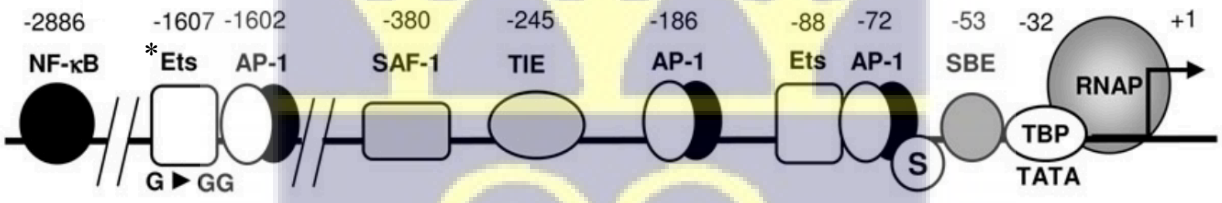
Figure 2.5: Schematic illustration of the pathway of MMP system through parturition. *Hormones, such as progesterone prostaglandin are regulated by uterine stretch, inflammatory cytokines, and corticotropin-releasing hormone (CRH) via the nuclear factor kappa B (NF-kB) pathway. Hormones such as prolactin, relaxin and urocortin as well as molecules such as; docosahexaenoic acid [DHA], eicosapentanoic acid [EPA] and mifepristone could also partake in the process to trigger MMP expression resulting in uterine and placental ECM remodelling to achieve parturition.*

Matrix metalloproteinases the main collagenase released by fibroblasts have been found to mediate the rupture of foetal membranes, both at term and preterm. According to Maymon and colleagues, *MMP-8* levels surge 50-fold in amniotic fluid during amnionitis, strongly linked to infection and preterm labour (Maymon *et al.,* 2001). Bacteria induce *MMP9* in foetal membranes with intrauterine leukocytes (Estrada-Gutierrez *et al.,* 2010). Certain bacteria like *Clostridium histolyticum* produce potent proteases degrading human collagen (Wu *et al.,* 2023).

2.18.3.1. The -1607 (G > GG) *MMP1* and SPTB

The *MMP1* gene is 8.2kb, 10 exon gene located at 102789919 to 102798160 (complement) on chromosome 11 (11q22.2) of the GRCh38.p14 assembly. Its enzyme, MMP1 or collagenase 1, is a major enzyme in interstitial collagens degradation. MMP1 is initially synthesized as an inactive proenzyme. Upon proteolytic activation, it hydrolyses a particular site collagen, leading to the generation of fragments. These fragments are susceptible to subsequent degradation by additional MMPs, mostly the gelatinases such as MMP2 and MMP9.

The *MMP1* gene promoter region is inundated with numerous regulatory elements that dictate its expression. **Figure 2.6** illustrates a schematic depiction of the transcription start site (indicated by the arrows at +1) as well as the response elements at positions in relation to the initiation of transcription.



Key: RNAP, RNA polymerase II complex; SBE, STAT-binding element; S, Smad-binding site; TBP, TATA binding protein; AP-1, Activator Protein-1; TIE, TGF-beta inhibitory element; SAF-1, serum amyloid A-activating factor-1; NF-kappaB-like binding site; Ets, E26 transformation-specific; *Ets; *MMP1* -1607 Ets binding site created by a G to GG polymorphism.

Figure 2.6: Schematic of MMP1 promoter cis-elements. *The MMP1 expression is complemented by functional consensus binding sites located relative to the transcription start site (shown by an arrow at +1). The Ets binding site has the G to GG polymorphism in the promoter region (Rowan & Young, 2007).*

All regulatory elements indicated in **Figure 2.6** play significant role in the expression of the *MMP1* gene. However, emphasis is placed on the *MMP1* -1607 Ets binding site in this study. A

single nucleotide polymorphism at nucleotide -1607 within the *MMP1* promoter involves the insertion of a guanine nucleotide (G). This insertion creates a central binding site (5'- GGAT-3') for Ets transcription factors which is associated with an elevation in *MMP1* promoter activity. Fujimoto *et al.* (2002) explored the functional significance of the polymorphism in *MMP1* expression in amnion cells and its link to PPROM. The 2G promoter exhibited over 2-fold higher activity than the 1G allele. Stimulation by phorbol 12-myristate 13-acetate (PMA) enhanced 2G allele binding and *MMP1* mRNA induction. Cells with 2G alleles produced more MMP1 protein upon PMA treatment, thereby establishing an association between 2G allele and PPROM risk (Fujimoto et al., 2002). However, in a case-control investigation involving 113 women with SPTB and 119 women with full-term delivery, Pereza *et al.* did not establish substantial proof supporting the link between *MMP1*-1607 1G/2G gene polymorphisms and SPTB among European Caucasian women (Pereza et al., 2014). Similarly, another study involving 255 cases and 255 controls in India did not find a significant association between *MMP1*-1607 1G/2G gene polymorphisms and SPTB (Pandey & Awasthi, 2020).

There is inter-racial differences in the distribution of *MMP1*-1607 (1G/2G) promoter genotypes. Fujimoto and colleagues found 23.8% 1G/1G, 47.2% 1G/2G, and 28.9% 2G/2G among 310 African American (Fujimoto et al., 2002). Rutter *et al.* also found 31% 1G/1G, 30% 1G/2G, and 39% 2G/2G from the Center d'Etude du Polymorphisme Humain (CEPH) population (Rutter *et al.,* 1998). In a study of 313 Japanese, Kanamori *et al.* had a genotype distribution 20% 1G/1G, 37.3% 1G/2G, and 42.7% 2G/2G (Kanamori *et al.,* 1999).

2.18.4. *AGTR2* and pregnancy

Angiotensin II receptor type 2 (*AGTR2*) gene is a 4.2 kilobase, 3 exon gene located at 116,170,744 to 116,174,974 on the X chromosome (Xq23) of the GRCh38.p14 assembly (accession number GCF_000001405.40). The gene encodes a G-protein coupled receptor, type-2 angiotensin II receptor (*AGTR2*) that binding angiotensin II (Mukoyama *et al.,* 1993). The cellular signalling via *AGTR2* remains unclear, but it possibly involves in cGMP and protein phosphatase activation, and the release of phospholipase A2 and arachidonic acid (Delforce et al., 2017). The *AGTR2* has been observed to be highly expressed in foetus, skin wound, and atretic ovary, indicating their role in growth and development. The *AGTR2* is also associated with a wide range of functions, including vasodilation, cellular apoptosis (Chow & Allen, 2016), natriuresis (Fatima *et al.,* 2021), synthesis of collagen (Königshoff *et al.,* 2007) and nitric oxide (Lemarié & Schiffrin, 2010).

The *AGTR2* (A1675G) and C4599A polymorphisms have been associated with the modulation uteroplacental circulation associated with preeclampsia, a major contributor to medically indicated preterm birth (Akbar *et al.,* 2009; Zhou *et al.,* 2013). According to Lanz *et al.,* (2003), *AGTR2* receptor– and Mitogen-Activated Protein Kinase (MAPK)-dependent mechanism provides the conduit for a reduction of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11-HSD2) by angiotensin II. The decreased activity of 11-HSD2 increases the intracellular availability of cortisol. The 11-HSD2 converts biologically active cortisol into inactive cortisone (Lanz *et al.,* 2003). Increased maternal serum cortisol levels during pregnancy is associated with pregnancy outcomes including medically indicated preterm birth via increased blood pressure or preeclampsia (Akbar *et al.,* 2009; Zhou *et al.,* 2013) and SPTB (Ijabi *et al.,* 2019).

2.18.4.1. *AGTR2* rs5950491 and gestational duration.

The association between *AGTR2* SNP (rs5950491) and gestational duration was first discovered by Zhang and colleagues in a in a two-stage GWAS involving Caucasian women. It is the first successful GWAS that discovered and replicated SNPs associated with spontaneous preterm birth and gestational duration. Other *AGTR2* SNPs were rs201226733, and rs5950506 (Zhang *et al.,* 2017). At the discovery stage of their study, Zhang and colleagues found each copy of the effect allele – the A allele of rs5950491 to shorten gestational duration by about 0.83 days ($p < 6.8 \times 10^{-11}$). This effect allele was observed to further shorten the gestational duration by 1.75 days ($p < 4.7 \times 10^{-8}$) at the replication stage using data from Nordic countries (Finland, Denmark, and Norway) of the study (Zhang *et al.,* 2017).

The SNP rs5950491 is positioned 157 kilobases upstream of *AGTR2* (**Figure 2.7**) implying that distal gene modulator might play a role in the molecular processes influencing the expression of the *AGTR2* gene. *AGTR2* expression decreases throughout pregnancy, reaching minimal levels in late pregnancy. This indicates that the regulation of *AGTR2* expression is both spatially and temporally controlled during the course of pregnancy (Delforce et al., 2017).

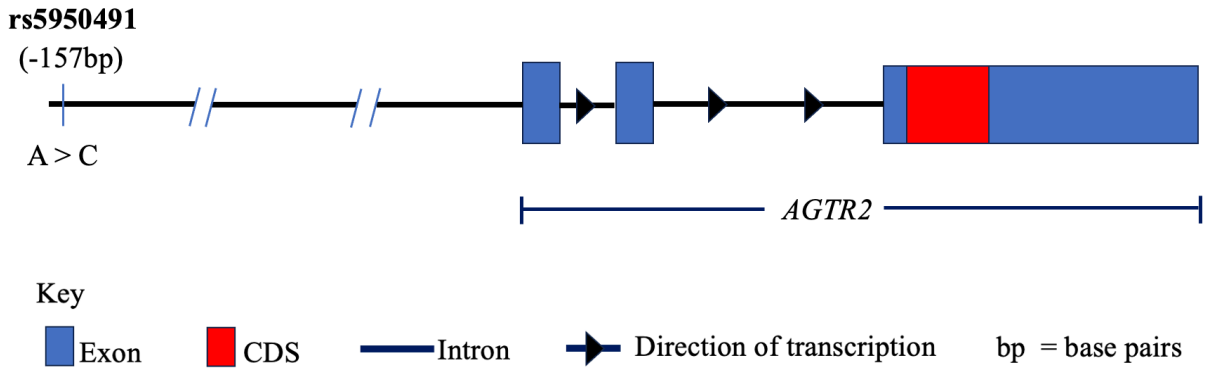
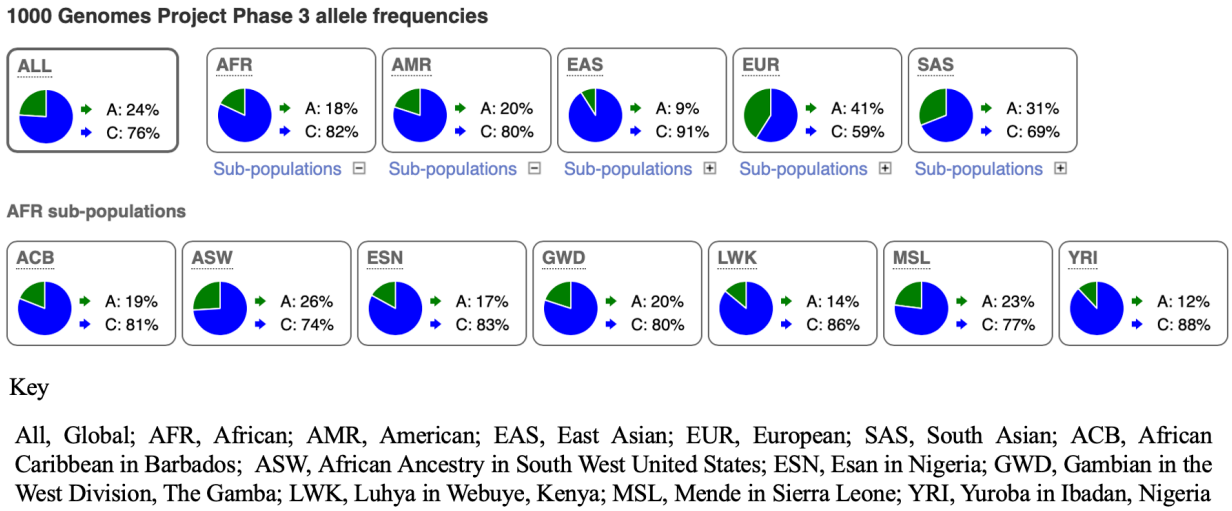


Figure 2.7: Schematic of AGTR2 gene structure showing (rs5950491). *The A > C polymorphism is located 157kb upstream of the AGTR2 gene. The blue and red rectangles represent the exons, the red rectangle alone represents the coding sequence (CDS). The black line are the introns and the black arrows illustrate the direction of transcription (not drawn to scale). Drawn with information from the National Centre for Biotechnology Information (NCBI) database (NCBI, 2023).*

According to National Centre for Biotechnology Information (NCBI) Allele Frequency Data for Variant Analysis and interpretation (ALFA) database, release version: 20230706150541, the reference allele (A) and the alternate (C) are detectable in the Africa population in the following proportion (A=0.2026, C=0.7974). Ensembl.org, also put the A allele frequency for the African population to be 0.181 and the C allele frequency to be 0.819 compared with the global A and C allele frequencies of 0.236 and 0.764 respectively. Peruvians have the least A allele frequency of 0.039 (Ensembl, 2024). **Figure 2.8** illustrates distribution of global and African distributions of the A and C alleles of rs5950491 copied from Ensembl.org (Ensembl, 2024).



Key

All, Global; AFR, African; AMR, American; EAS, East Asian; EUR, European; SAS, South Asian; ACB, African Caribbean in Barbados; ASW, African Ancestry in South West United States; ESN, Esan in Nigeria; GWD, Gambian in the West Division, The Gamba; LWK, Luhya in Webuye, Kenya; MSL, Mende in Sierra Leone; YRI, Yuroba in Ibadan, Nigeria

Figure 2.8: Global and African sub-distribution of A and C alleles of rs5950491.
The global A and C alleles frequency of the rs5950491 from the 1000 genomes phase 3 (ALL) is 0.24 and 0.76 respectively. East Asians have the least minor allele (A) frequency of 0.09. Among the African sub-population, the A and C allele frequencies varied from (A = 0.26 and C = 0.74) among African Ancestry in South West United States (ASW) to (A = 0.12 and C = 0.88) among the Yuroba in Ibadan, Nigeria (YRI).

2.18.5. *WNT4* and pregnancy

The Wingless-type Mouse Mammary Tumour Virus integration site family member 4 (*WNT family member 4* or *WNT4*) gene is a 25.7 kilobase, 5-exon gene located at 22117313 to 22143097 (complement) on chromosome 1 (1p36.12) of the GRCh38.p14 assembly (accession number GCF_000001405.40). *WNT4* is one of the 19 Wnt family of secreted hydrophobic, cysteine-rich glycoproteins secreted into the extracellular environment in mammals. They are initially produced as precursor proteins, comprising a brief N-terminal signal sequence and a mature segment with varied length, typically ranging from around 320 to 400 amino acids. The WNT signalling is highly conserved and possesses the capacity to initiate signalling through either the canonical or noncanonical pathways as demonstrated in **Figure 2.9** and **Figure 2.10,** respectively.

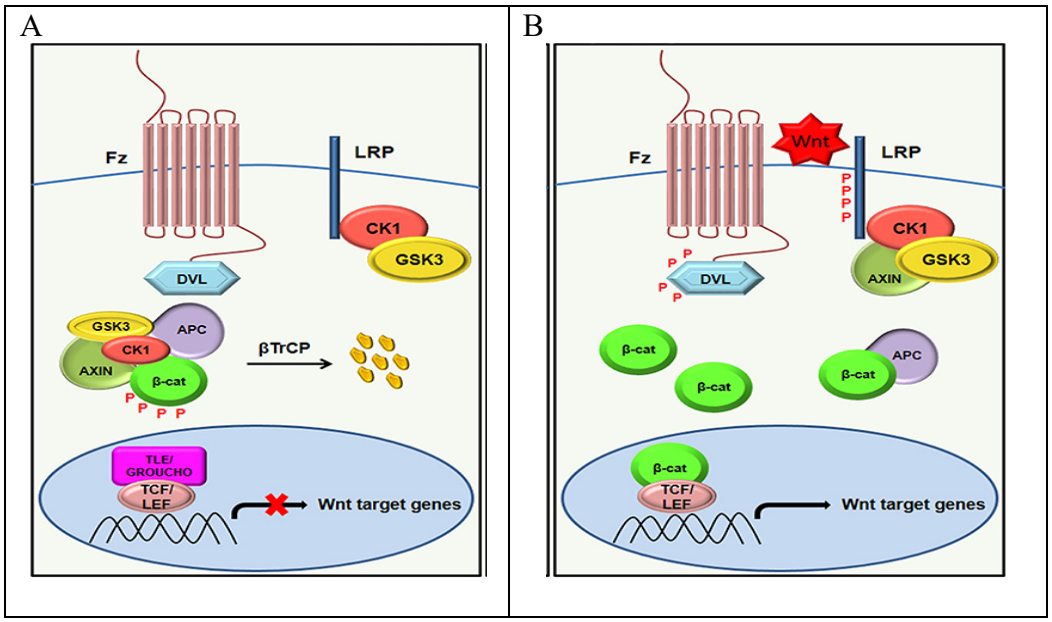


Figure 2.9: Canonical Wnt-signalling pathway (Patel et al., 2019). *In the absence of a Wnt ligand (**Figure 2.9, panel A**), the destruction complex, comprising axin, Adenomatous Polyposis Coli (APC), Casein Kinase 1 (CK1), and Glycogen Synthase Kinase 3 beta (GSK3β), phosphorylates β-catenin leads to β-catenin's ubiquitination by Beta-Transducin Repeat Containing E3 Ubiquitin Protein Ligase (β-TrCP), marking it for proteasomal degradation. This absence in the nucleus prompts the binding of a repressor complex with transcription factors T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) and Transducin-Like Enhancer of Split (TLE)/Grouche, suppressing target gene activity. Conversely, with Wnt engagement (**Fig. 2.9, panel B**), CK1 and GSK3β phosphorylate Low-Density Lipoprotein Receptor-related Protein receptors (LRP) receptors. This recruit Dishevelled (Dvl) proteins to the plasma membrane, activating and organizing the β-catenin destruction complex. Resultantly, β-catenin accumulates in the cytoplasm, then translocates into the nucleus to form a complex with TCF/LEF, initiating target gene transcription (Patel et al., 2019).*

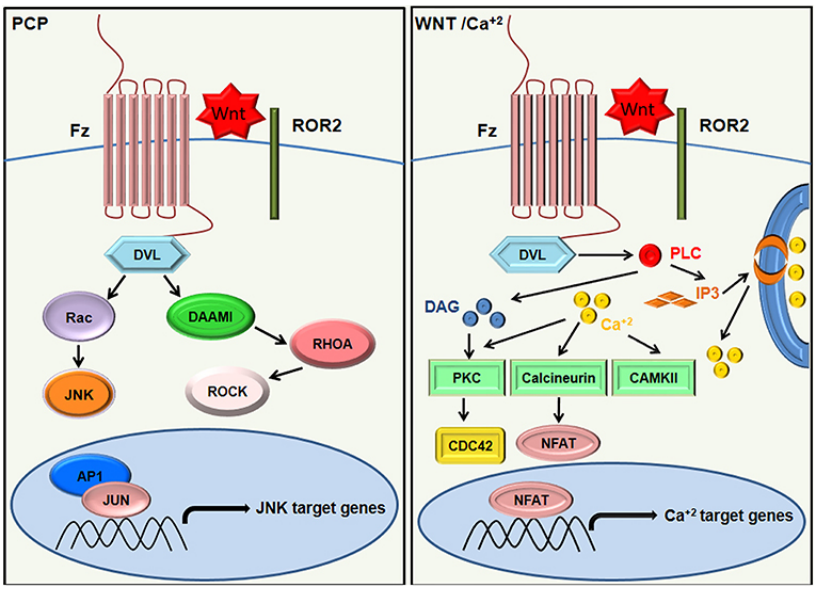
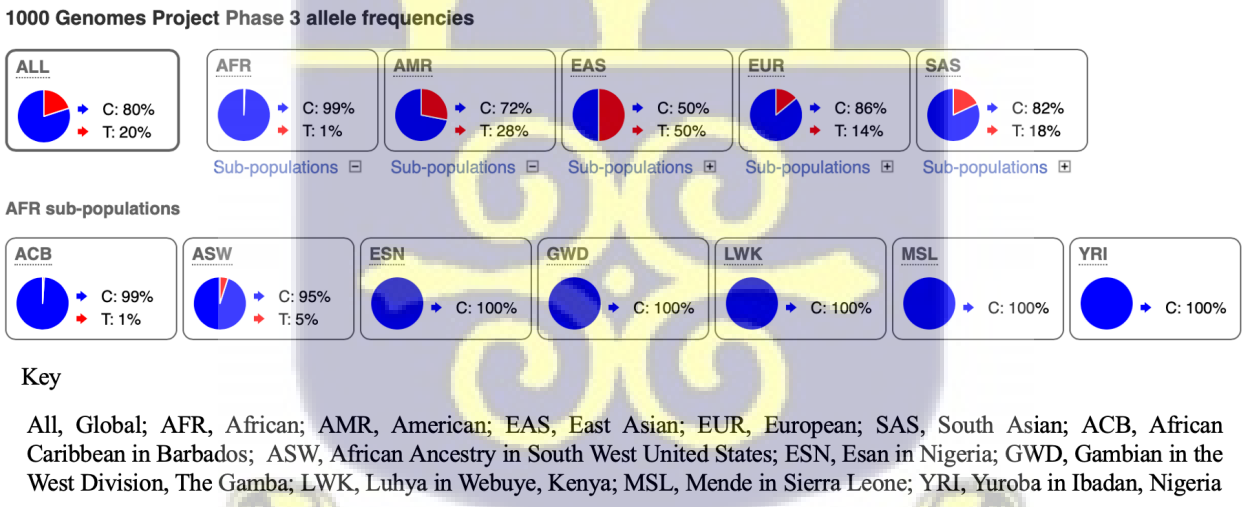


Figure 2.10: Non canonical Wnt-signalling pathway (Patel et al., 2019). *In the non-canonical Wnt/Planar Cell Polarity (PCP) pathway (**Figure 2.10, panel A**), Wnt ligand binding to the ROR-Frizzled receptor complex triggers Dishevelled (Dvl) activation. This activates Rho by releasing inhibition on Dishevelled-Associated Activator of Morphogenesis (DAAM), collaborating with Ras-Related C3 Botulinum Toxin Substrate 1 (Rac1). Together, they stimulate Rho-Associated Coiled-Coil Kinase (ROCK) and c-Jun N-terminal Kinase (JNK), driving polarized cell migration. Conversely, in the WNT/$^{Ca+2}$ pathway (**Figure 2.10, Panel B**), Phospholipase C (PLC) activation generates Diacylglycerol (DAG) and Inositol trisphosphate (IP3). This induces intracellular calcium fluxes, activating distinct Protein Kinase C (PKC) isoforms and calcium-modulated kinases (CAMKII). These activated PKC isoforms and CAMKII trigger a Nuclear Factor of Activated T (NFAT)-dependent transcriptional response (Patel et al., 2019).*

The Wnt pathway, is vital in development, cell regulation, and tissue maintenance. However, the non-canonical Wnt pathways still needs a lot of work to be well characterized due to the diverse receptors and downstream effectors involved. (Ishitani *et al.,* 2003; Olson & Gibo, 1998). Wnt signalling is crucial for embryonic development. Complete loss of *WNT4* function during embryonic development could be lethal, especially, due to the impeded metanephric kidney (functional kidney) development (Meng *et al.,* 2020).

Enhanced *WNT4* expression induced by oestrogen in pregnancy regulates endometrial stromal cell proliferation, survival, and differentiation, which are crucial for embryo support and successful pregnancy (Patel *et al.,* 2019; Sonderegger *et al.,* 2010). The consequences of SNPs in the *WNT4* gene, however, could be pleiotropic, ranging from the proliferation of cells in endometrial fibroblasts, resulting in effective implantation and elongation of gestational duration, to endometriosis (the abnormal growth of tissue similar to the lining of the uterus, or endometrium, outside the uterus) and cancers (Clevers, 2006; Pavlicev *et al.,* 2022; Pitzer *et al.,* 2021) Reduced *WNT4* expression in the uterus has been associated with impaired decidualization and results in preeclampsia, a major risk factor in preterm birth (Wang *et al.,* 2016). **Figure 2.11** illustrates distribution of global and African distributions of the C and T alleles of rs56318008 copied from Ensembl.org (Ensembl, 2024).



Key

All, Global; AFR, African; AMR, American; EAS, East Asian; EUR, European; SAS, South Asian; ACB, African Caribbean in Barbados; ASW, African Ancestry in South West United States; ESN, Esan in Nigeria; GWD, Gambian in the West Division, The Gamba; LWK, Luhya in Webuye, Kenya; MSL, Mende in Sierra Leone; YRI, Yuroba in Ibadan, Nigeria

Figure 2.11: Global and African sub-distribution of C and T alleles of rs56318008.*The global C and T alleles frequency of the rs56318008 from the 1000 genomes phase 3 (ALL) is 0.80 and 0.20 respectively. Africans have the least minor allele (T) frequency of 0.01. compared with the global frequency. Generally, the African sub-population has the C and T allele frequencies varying from (C = 0.95 and T = 0.05) among African Ancestry in South West United States (ASW) to (T = 0.00 and C = 100) among the ESN, GWD, LWK, MSL, YRI.*

2.18.5.1. *WNT4* rs56318008 and gestational duration

The rs56318008, alleles (C > T) is located 817 bases upstream of the *WNT4* gene, chr1:22143914 (GRCh38.p14) of the NCBI *Homo sapiens* GRCh38.p14 as shown in the sketch in **Figure 2.12**

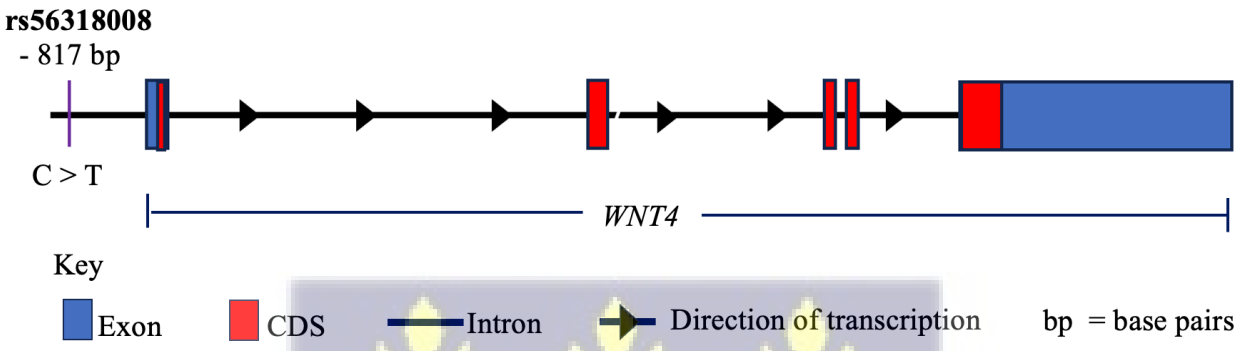


Figure 2.12: Schematic of the WNT4 gene showing rs56318008. *The C > T polymorphism is located 817 upstream of the AGTR2 gene. The blue and red rectangles represent the exons, the red rectangle alone represents the CDS. The black line are the introns and the black arrows illustrate the direction of transcription (not drawn to scale). Drawn with information from the National Centre for Biotechnology Information (NCBI) database (NCBI, 2023).*

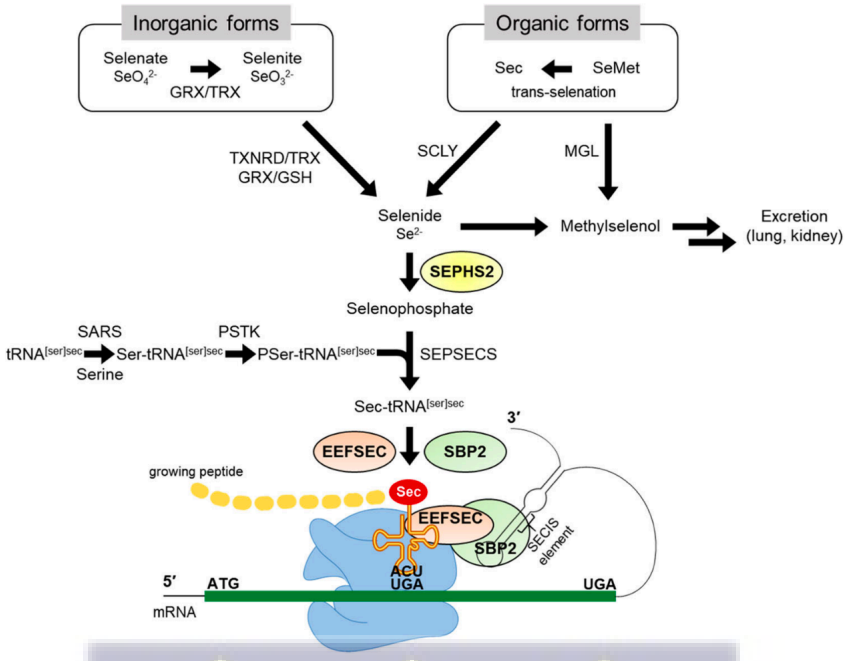
The association between rs56318008 and gestational duration was discovered by Zhang and colleagues, 2017 in a GWAS to determine genetic markers associated with SPTB and gestational duration. The SNP was found to increase gestational age by 2 days, hence protective against SPTB (Zhang *et al.,* 2017). The T allele of rs56318008 had a frequency of 0.139, effect size of 1.05 (increased gestational duration by about 1.05 day, $p < 1.2 \times 10^{-9}$) in the European cohort that was used in the discovery phase. In the replication phase, the T had a frequency of 0.153, effect size of 2.27 (increased gestational duration by about 2.27 day, $p < 1.8 \times 10^{-7}$).

Works on the association between rs56318008 and gestational duration is scanty. However, a couple of works have been done to assess its relationship with some gynaecological pathologies.

In a Chinese case/control study involving 707 epithelial ovarian cancer (EOC) patients and 1563 unrelated controls the T allele of rs56318008 has been found to be associated with EOC [aOR 1.28 (1.13–1.45), $p < 0.000645$] (Zhang *et al.,* 2018). A similar case control study where the rs56318008 was genotyped in 930 women with endometriosis and 959 controls found the rs56318008 to increase the risk of endometriosis by almost 36% fold, OR 1.36 (95% CI: 1.15-1.62, $p < 3.86 \times 10^{-4}$) (Luong *et al.,* 2013).

2.18.6. *EEFSEC* and pregnancy

The selenocysteine tRNA specific eukaryotic elongation factor gene (*EEFSEC*) is a 0.26 mega base gene with 7-exon and located at 128153481 to 12840646 on chromosome 3 (3q21.3) of the GRCh38.p14 assembly (accession number GCF_000001405.40). *EEFSEC*, a eukaryotic elongation factor facilitates the insertion of the amino acid selenocysteine into nascent selenoproteins during translation in protein synthesis. EEFSEC interacts with a specialized transfer RNA (tRNA) molecule known as tRNA[Ser]Sec, which carries selenocysteine (Sec). This interaction helps guide the selenocysteine-loaded tRNA mediated by Selenocysteine Insertion Sequence (SECIS) binding protein 2 [SBP2] to the A-site of ribosome and binds to SECIS located in the 3′UTR of a selenoprotein mRNA. The UGA codon is subsequently recognized as the Sec integration codon for the incorporation of the selenocysteine into a polypeptide to synthesise selenoproteins as shown in **Fig. 2.13**.



Key: SCLY, Selenocysteine Lyase; MGL, Methylglyoxalase; SeMet, Selenomethionine; GRX, Glutaredoxin; TRX, Thioredoxin; TXNRD, Thioredoxin Reductase; GSH, Glutathione; SARS, Selenocysteine Transfer RNA (tRNA)-Specific Adenosine Deaminase; PSTK, Phosphoseryl tRNA Kinase; SEPSECS, O-phosphoseryl-tRNA(Sec) selenium transferase; SBP2, Selenocysteine Insertion Sequence binding protein 2

Figure 2.13: Eukaryotic selenium metabolism. *The inorganic forms are reduced by either Thioredoxin Reductase (TXNRD) or Thioredoxin (TRX) as well as Glutaredoxin (GRX) or Glutathione (GSH). The organic forms are cleaved by selenocysteine lyase (SCLY), both producing selenide. The selenide is converted into Selenophosphate by selenophosphate synthetase 2 (SEPHS2). A subsequent reaction between Selenophosphate and PSer-tRNA[Ser]Sec catalysed by O-phosphoseryl-tRNA(Sec) selenium transferase SEPSECS yields Sec-tRNA[Ser]Sec. The selenocysteine tRNA specific eukaryotic elongation factor (EEFSEC) and Selenocysteine Insertion Sequence binding protein 2 (SBP2) which is bound to Selenocysteine Insertion Sequence (SECIS) of the 3'UTR of a selenoprotein mRNA mediate the transfer of Sec-tRNA[Ser]Sec to the A-site of ribosome. The UGA codon thus become a selenocysteine (Sec) integration codon (Kang et al., 2020).*

Selenoproteins, like glutathione peroxidases and thioredoxin reductases, play crucial roles in cellular redox balance, antioxidant defence, and controlling inflammation (Aderao *et al.,* 2023; Golin *et al.,* 2023; Labunskyy *et al.,* 2014; S. Li *et al.,* 2020). Selenoprotein 5'deiodinases are essential for thyroid hormone function, impacting growth and energy metabolism (Mullur *et al.,*

2014). These are critical for cellular homeostasis and overall health. Additionally, maternal physiological states including her redox status, inflammation and energy metabolism have been found to increase preterm birth risk (Burnum *et al.,* 2012; Chen *et al.,* 2015).

The benefits of selenium and its products in pregnancy outcomes have been identified from many studies (Barman et al., 2020). In a Norwegian prospective population-based study involving 72,025 women, maternal dietary Se intake was associated with reduced preterm delivery risk by 8%, hazard ratio = 0.92, 95 % CI: [0.87 – 0.98], but Se supplements and blood Se status showed no such associations (Barman *et al.,* 2020). Moreover, Malawi, a country with one of the highest preterm birth prevalence in the world (18%) has over 80% of the population at risk of selenium deficiency (Hurst *et al.,* 2013).

The impact of dysfunctional *EEFSEC* on the overall cellular function is even much more deleterious than selenium deficiency. It is associated with phenotypes like dilated cardiomyopathy, axial muscle weakness, scoliosis and seizures (Dubey & Copeland, 2016; Fradejas-Villar, 2018). **Figure 2.14** illustrates distribution of global and African distributions of the A and G alleles of rs2955117 copied from Ensembl.org (Ensembl, 2024).

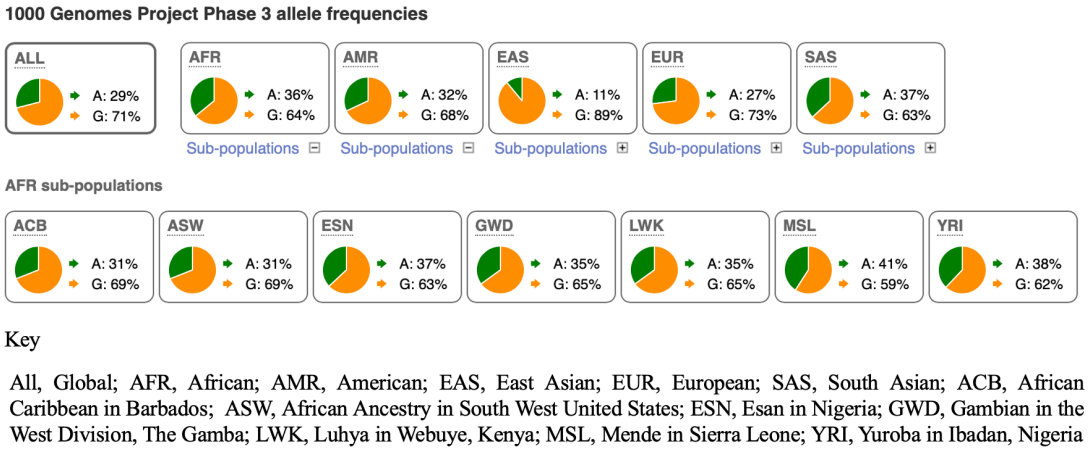


Figure 2.14: Global and African sub-distribution of G and A alleles of rs2955117. *The global G and A alleles frequency of the rs2955117 from the 1000 genomes phase 3 (ALL) is 0.73 and 0.29 respectively. Africans have G and A alleles frequencies of 0.64 and 0.36 respectively. Among the African sub-population, the G and A allele frequencies varied from (G = 0.59 and A = 0.41) among the MSL to (G = 0.63 and A = 0.37) among the ASW.*

2.18.6.1. *EEFSEC* rs2955117 and gestational duration

The rs2955117, alleles (G > A) is an intronic variant located between the first and the second introns of the *EEFSEC* gene, chr 3:128162770 (GRCh38.p14) of the NCBI Homo sapiens as shown in the sketch in **Figure 2.15.**

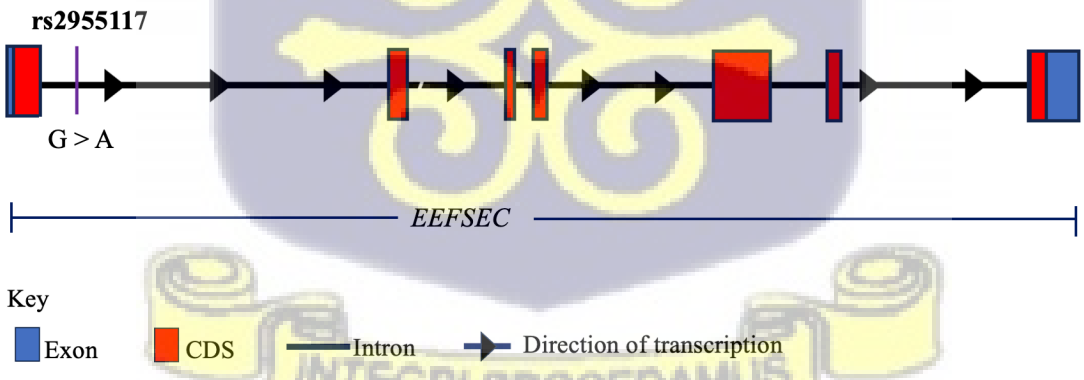


Figure 2.15: A schematic of the EEFSEC gene showing rs2955117. *The rs2955117 is situated between the first and second exons. The blue and red rectangles represent the exons, the red rectangle alone represents the CDS. The black line are the introns and the black arrows illustrate the direction of transcription (not drawn to scale). Drawn with information from the National Centre for Biotechnology Information (NCBI) database (NCBI, 2023).*

The association between rs2955117 and gestational duration was discovered by Zhang and colleagues, 2017 in a GWAS to determine genetic markers associated with SPTB and gestational duration. The SNP was found to increase the risk of SPTB (Zhang *et al.,* 2017). The rs2955117 had a frequency of 0.286, effect size of 0.91 (increased gestational duration by about 0.91 day) and a p-value of 7.2×10^{-12} in the European cohort that was used in the discovery phase. In the replication phase, the A allele had a frequency of 0.279, had an effect size of 1.33 (increased gestational duration by about 1.33 day) ($p < 1.6 \times 10^{-4}$).

Afterwards, a nested case-control study involving 541 UK women was conducted to determine whether maternal serum Se deficiency and rs2955117 of the *EEFSEC* gene concurrently increase the risk of SPTB. It was a found that serum selenium level < 83.3 ppm in the second trimester increases the risk of SPTB, RR = 2.7, 95% CI: [1.5 – 4.8]; p = 0.001) compared with the controls. However, a targeted analysis of the rs2955117 of the *EEFSEC* gene did not indicate an association with preterm birth, OR = 0.95, 95% CI: [0.59 – 1.56]; p = 0.82) (Care *et al.,* 2021).

2.18.7. *ADCY5* and pregnancy

Adenylyl cyclases (ADCs) are a molecular family of transmembrane proteins that participate in the conversion of adenosine triphosphate (ATP) into cyclic adenosine-3′,5′-monophosphate (cAMP), which serves as a secondary messenger that mediates diverse downstream signalling through cAMP-binding proteins such as cAMP-dependent kinases, ion transporters and transcription factors (Ferrini *et al.,* 2021) as shown in **Fig. 2.16.**

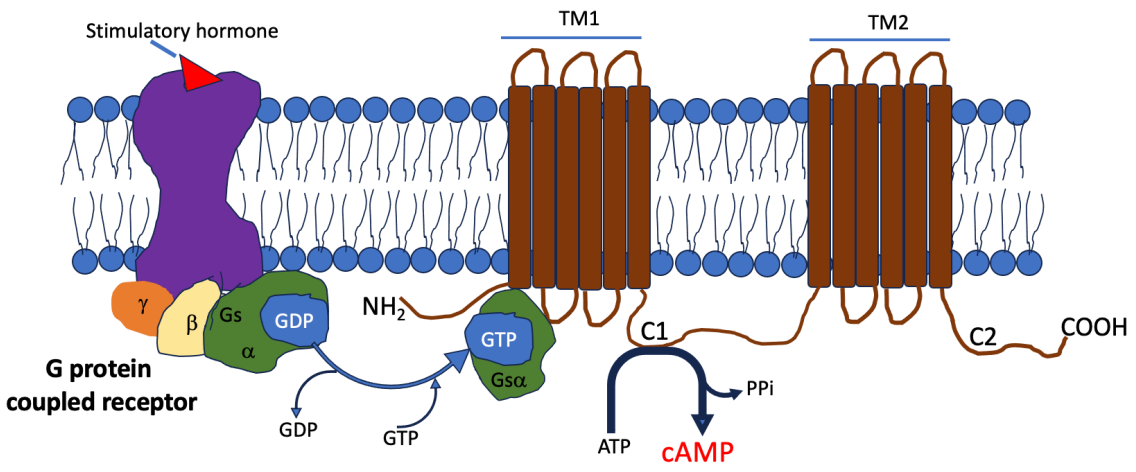
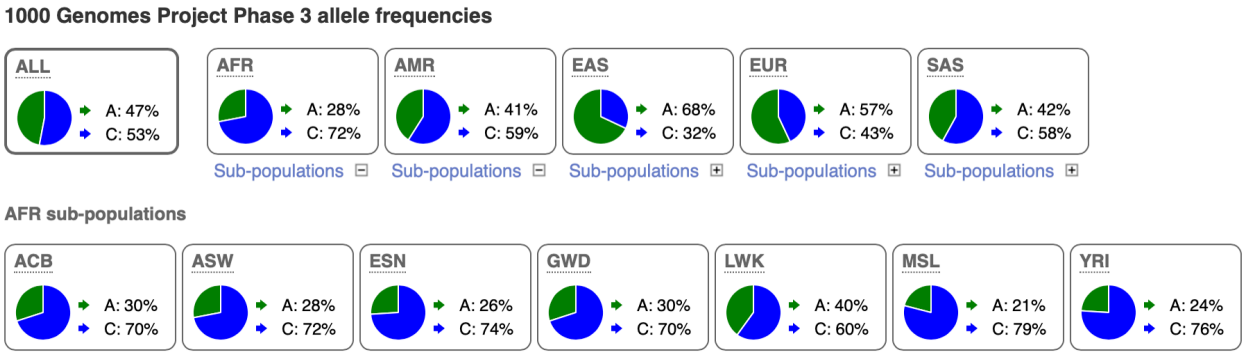


Figure 2.16: A schematic adenylyl cyclase proteins in G protein coupled signalling. *It comprises a pair of six transmembrane (TM) helices domains with two intracellular catalytic domains (C1 and C2), regulated by G proteins that are coupled to membrane receptors. An activation of the receptor prompts a conformational shift, leading the α subunit of the G protein complex to separate and bind guanosine triphosphate (GTP). Gα-GTP activates adenylyl cyclase, which converts ATP into cAMP (Devasani & Yao, 2022).*

Isoform 5 of adenylyl cyclase (*ADCY5*) is expressed in several tissues including the endometrium. The *ADCY5* is a 0.17 mega base, and 21-exon gene located at 123282296 to 123449090 (complement) on chromosome 3 (3q21.1) of the GRCh38.p14 assembly (accession number GCF_000001405.40). *ADCY5* has been found to be highly expressed in the placentas of women with gestational diabetes mellitus (GDM), a pregnancy related metabolic disorder and a major attributable risk factor to preterm birth compared with that of healthy women (Arora *et al.,* 2018; Ustianowski *et al.,* 2021). SNPs in *ADCY5* have been found to decrease beta-cell function [Homeostatic Model Assessment (HOMA)-indices of beta-cell function], reducing foetal insulin, a major foetal growth factor, resulting in reduced birth weight (Freathy *et al.,* 2010; Lin *et al.,* 2020). **Fig. 2.17** illustrates distribution of global and African distributions of the C and A alleles of rs9861425 obtained from Ensembl.org (Ensembl, 2024).



Key

All, Global; AFR, African; AMR, American; EAS, East Asian; EUR, European; SAS, South Asian; ACB, African Caribbean in Barbados; ASW, African Ancestry in South West United States; ESN, Esan in Nigeria; GWD, Gambian in the West Division, The Gamba; LWK, Luhya in Webuye, Kenya; MSL, Mende in Sierra Leone; YRI, Yuroba in Ibadan, Nigeria

Figure 2.17: Global and African sub-distribution of C and A alleles of rs9861425. *The global C and A alleles frequency of the rs9861425 from the 1000 genomes phase 3 (ALL) is 0.53 and 0.47 respectively. Africans have C and A alleles frequencies of 0.72 and 0.28 respectively. Among the African sub-population, the C and A allele frequencies varied from (C = 0.60 and A = 0.40) among the LWK to the MSL with the least minor allele frequency (C = 0.79 and A = 0.21).*

2.18.7.1. *ADCY5* rs9861425 and gestational duration

The rs9861425, alleles (A > C) is an intronic variant located between the first and the second introns of the *ADCY5* gene, chr 3:123354036 of the NCBI *Homo sapiens* GRCh38.p14 as shown in the sketch in **Fig. 2.18.**

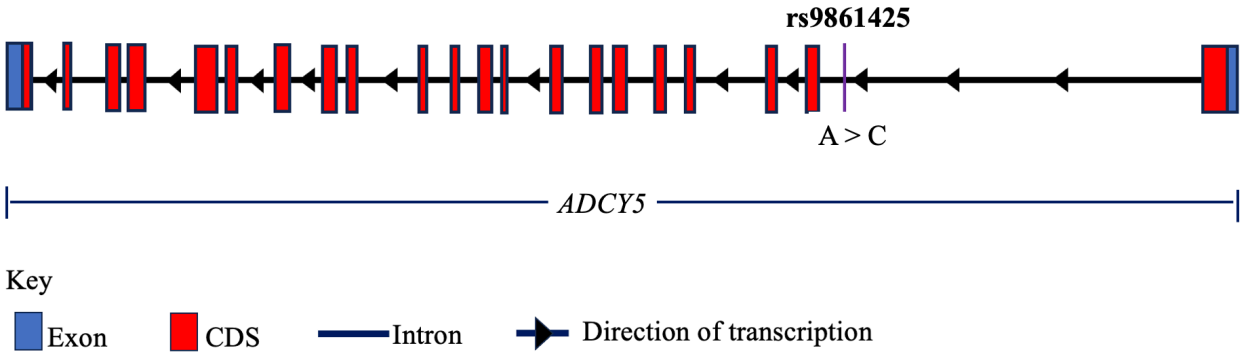


Figure 2.18: A schematic of the ADCY5 gene showing rs9861425. *The rs9861425 is situated between the first and second exons. The blue and red rectangles represent the exons, the red rectangle alone represents the CDS. The black line are the introns and the black arrows illustrate the direction of transcription (not drawn to scale). Drawn with information from the National Centre for Biotechnology Information (NCBI) database (NCBI, 2023).*

Zhang and colleagues first identified rs9861425 as one of the SNPs associated with gestational duration in a GWAS to determine genetic markers associated with SPTB and gestational duration. The SNP was found to reduce the risk of SPTB (Zhang *et al.,* 2017). The C allele of rs9861425 had a frequency of 0.453, effect size of -0.60 (decreased gestational duration by about 0.6 days, 6.1 x 10^{-07}) in the European cohort that was used in the discovery phase. In the replication phase, the C alleles had a frequency of 0.470, effect size of -1.38 (decreased gestational duration by about 1.38 days, p-value of 9.5 x 10^{-6}). Works on the association between rs9861425 and pregnancy in other populations, especially Blacks is very scanty.

2.19. Maternal knowledge of personal and immediate family birth history.

History of preterm delivery is a principal predictor of SPTB (Zhang *et al.,* 2015). Recurrent preterm births are increased by over 2-fold among women who themselves were born preterm (Smid *et al.,* 2017; J. Yang *et al.,* 2016). Previous SPTB increases subsequent ones by 4-fold (Hammond *et al.,* 2013; Mercer *et al.,* 1999; Smid *et al.,* 2017; Yang *et al.,* 2016). Maternal

knowledge of whether she was born preterm or term as well as the knowledge of the birth history of the immediate family members (mother and sister(s)) is a therefore crucial to mothers' preparedness for SPTB. This is especially important due to the sudden occurrence of SPTB as well as the reduced infrastructure in developing countries like Ghana.

Unfortunately, information about mothers with SPTB's awareness of their birth history regarding preterm birth in Africa and in particular Ghana is very scanty.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Ethical clearance

The study was approved by the following institutions: Ghana Health Service Ethics Review Committee (GHS-ERC 009/02/21), Korle Bu Teaching Hospital Institutional Review Board (KBTH-IRB/00003/2020), Komfo Anokye Teaching Hospital Institutional Review Board (KATH-IRB/AP/022/20), Ethics Committee for Basic and Applied Sciences (ECBAS 007/19-20), University of Ghana. Further permission was sought from the management of the selected health facilities. Individual informed consent was conducted in the language of the participant and documented prior to enrolment of potential participants willing to participate in the study.

3.2. Study population and location of research

The study population was immediate puerperium mothers with singleton SPTB neonates (cases) and mothers with singleton term neonates (controls) receiving obstetric care at the obstetrics and gynaecology departments of six health facilities at different parts of Ghana as shown in **Figure 3.1.**

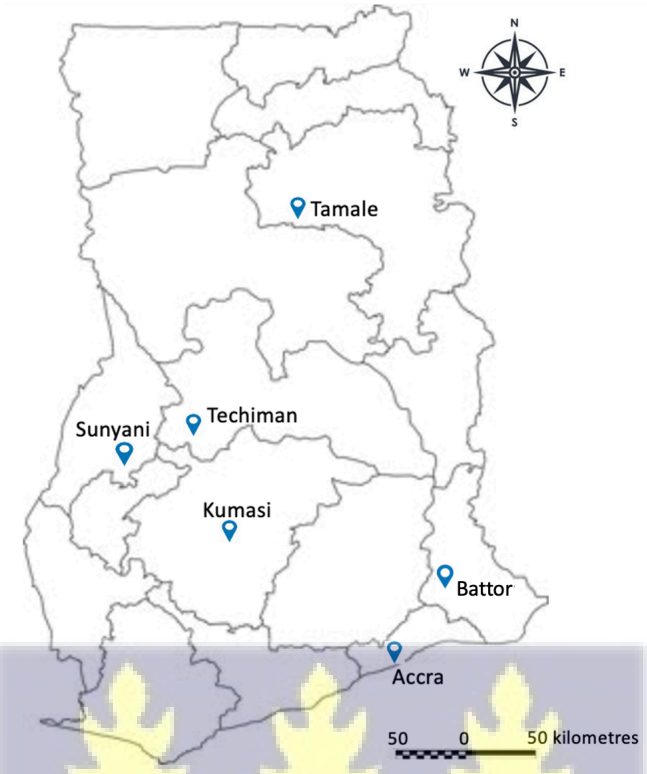


Figure 3.1: Distribution of the study centres in Ghana. *The distribution of the study centres was targeted to substantially capture the sociocultural behaviours and ethnic groups in Ghana (Ganle, 2016). Three of the hospital namely: Korle-Bu Teaching Hospital, Accra; Komfo Anokye Teaching Hospital, Kumasi; and Tamale Teaching Hospital, Tamale situated in the southern, middle belt and northern parts of Ghana, respectively are tertiary hospitals that receive referred clients from all parts of the country. Three secondary facilities namely: Bono Regional Hospital, Sunyani; and Holy Family Hospital, Techiman and Battor Catholic hospital were included.*

3.3. Study design and sampling technique

A case-control study design was used. A convenience sampling method was adopted to enrol women who accepted to take part in the study and met the inclusion criteria. Immediate puerperium mothers with singleton SPTB neonates (cases) and mothers with singleton term neonates (controls) attending five health facilities strategically chosen to increase the chances of ethnic and sociocultural diversity were approached and screened for illegibility using the study inclusion criteria. Mothers who met the inclusion criteria and were willing to participate were

taken through informed consent documentation and subsequently enrolled into the study. The goal was to collect whole blood and placental samples from all participants.

3.4. Exclusion and inclusion criteria

Clinical data was utilised and interviews conducted to exclude participants with chronic health conditions such as hospital recorded vaginal infection, pregnancy-induced hypertension, thyroid-related disease, pre-eclampsia, mental disorders, pre-gestational/gestational diabetes, heart-related disease, HIV or AIDS, minor and major foetal malformations, lupus, uterine malformations, rheumatoid arthritis, Crohn's disease, ulcerative colitis, cancer and influenza. Additionally, mothers with multiple gestations, with medical conditions of pregnancy requiring induction of labour and those with lifestyle habits including alcoholism, cigarette smoking and recreational drug use during pregnancy were excluded.

Generally, all pregnancies included were intrauterine. Labour involved regular, painful uterine contractions, progressive cervical effacement and dilatation (American College of Obstetricians and Gynecologists, 2014). The controls had gestational weeks between 37 to 42. The SPTB had gestation weeks less than 37 but not less than 24 weeks with life neonates and declared by an obstetrician and gynaecologist or a midwife as a spontaneous preterm birth. Mothers with recorded ultrasound examination which was within the first trimester of pregnancy were used (American College of Obstetricians and Gynecologists, 2014).

3.5. Clinical demographic data collection

A spreadsheet with codified unique identifiers for each participant was used for recording individual participant information (Retshabile *et al.,* 2018). Maternal clinical and demographic

characteristics were obtained from the participant's health record. These characteristics included: age, blood pressure, blood haemoglobin level at booking, type of preterm birth (spontaneous preterm labour [SPTL] or preterm premature rupture of membrane [PPROM]), marital status, height, and parity. Neonatal characteristics including: age, length, gestational age, and first- and fifth-minute Apgar scores were also recorded their health records.

A structured questionnaire was used to capture mother's ethnicity, age at menarche and highest educational level. Missing data in the health record were obtained through interview or during follow-up telephone interviews.

3.6. Neonate anthropometry and Apgar score measurement

The neonates' weight, length and head circumference were taken within the first one hour after birth. The weights were measured using an electronic weighing scale (Seca®) and the weight was recorded to the nearest 100th of a kilogram. All the scales used were standardised and checked daily for errors. The length was measured to the nearest centimetre with the use of an infantometre. The neonate was carefully laid flat on the infantometre and the length was carefully recorded by positioning the device from the crown to heels, making sure that it is in contact with the body without compression (Surkan et al., 2017). The head circumference was measured by placing a non-stretchable flexible measuring tape around occipitofrontal circumference of the neonate's skull. Measurement was recorded in the nearest centimetre (Surkan et al., 2017).

The first- and fifth-minute Apgar scores were recorded by trained midwives and paediatricians.

3.7. Maternal Depression assessment

The 20 questions Centre for Epidemiological Studies – Depression (CES-D) scale was used to measure maternal psychological state in the 7 days prior to the interview and scored accordingly (Radloff, 1977). Four of the questions (4, 8, 12, and 16) were scored from 3 to 0. The remaining questions (1, 2, 3, 5, 6, 7, 9, 10, 11, 13, 14, 15, 17, 18, 19, 20) were scored from 0 to 3. The scores were then summed to screen for persons with depressive disorder. A score of 16 and above indicated depression, a score lower than 16 suggested no depression. The scores were further categorised into: "No depression" (CES-D score <16), "Possible depression" ($16 \geq$ CES-D score ≤ 19), "Depression" ($20 \geq$ CES-D score ≤ 24), "Severe depression" (CES-D score ≥ 25) (Wemakor & Mensah, 2016).

3.8. Maternal knowledge on personal and immediate family preterm birth status

An interview guide was used to assess mothers' awareness of whether they were born preterm, whether their mothers were born preterm, and whether their first-degree sisters (if any) were born preterm. Preterm research in Ghana has primarily focused on hospital data. This was a preliminary study conducted to ascertain the women's knowledge of their personal birth history as well as that of their immediate family. The outcome is to guide further studies on community involvement in preterm birth research.

3.9. Tissue Sample collection

Four millilitres of venous blood were collected from the participants via venepuncture into EDTA-vacutainer tubes under strict aseptic conditions by either a trained phlebotomist, a midwife, or a nurse. A pathologist also took two placental biopsies for the study. These were 0.5

cubic centimetres of placental tissue taken from the maternal side of the placenta and fixed in QIAGEN™ RNA later (Qiagen, Hilden, Germany) and 5 cubic centimetre piece of placental tissue taken from the maternal side of the placenta and fixed in fixed in 10% buffered formalin. All the samples were labelled with the participant's unique identifiers used for the clinical and demographic data collection. All the samples apart from the sample in buffered formalin were transported on dry ice to the laboratory at the West African Centre for Cell Biology of Infectious Pathogens, Department of Biochemistry Cell and Molecular, University of Ghana and stored at -80°C prior to laboratory analysis.

3.10. Placental histology assessment

The 10% formalin fixed placental tissues were dehydrated by placing them through a series of graded ethanol solutions (70%, 80%, 95%, and 100%) for five minutes each to remove water. The tissues were cleared by immersing them in xylene, repeated twice for 10-15 minutes each time (Nijman *et al.,* 2016). The tissues were then infiltrated with molten paraffin wax at 60°C overnight and embedded in paraffin blocks. Each of the paraffin-embedded tissues was mounted onto a microtome and sections (5 µm thick) were cut. The tissue sections were transferred onto glass slides and placed in xylene for 5 minutes to remove the paraffin wax. The slides were transferred to a series of graded ethanol solutions (100%, 95%, 80%, and 70%) at 3 minutes per tissue section for rehydration, immersed in hematoxylin stain for 10 minutes and rinsed with distilled water to remove excess hematoxylin. The slides were dipped in acid alcohol for 30 seconds to differentiate the staining and checked under the microscope to ensure adequate differentiation (Nijman *et al.,* 2016). Subsequently, the slides were immersed in eosin stain for 2 minutes and rinsed with distilled water to remove excess eosin. They were then dehydrated in a

graded series of ethanol solutions (70%, 95%, and 100%) at 2 minutes each. The tissues on the slides were cleared by dipping them in xylene for 5 minutes to remove the ethanol from the tissue to enhance infiltration of paraffin wax. Coverslips were applied to each slide using Canada balsam to preserve the stained tissue. The stained placental tissue sections were examined under a light microscope by a blinded trained pathologist without knowledge of the clinical data associated with each placental tissue Placental lesions were designated based on the nomenclature endorsed by the Amsterdam Placental Workshop Group (Khong *et al.,* 2016).

3.11. Genotyping

3.11.1. DNA extraction and quantification

Whole blood collected from maternal peripheral venous blood was used to extract genomic DNA using Qiagen DNA extraction kit (Qiagen, Hilden, Germany) based on manufacturers protocol. The heat block and elution buffer were equilibrated to 56 °C and room temperature 25ºC respectively. The venous blood of 100 µl was transferred into a 2 ml micro-centrifuge tube, 20 µl of proteinase K and 200 µl of buffer AL (Tissue Lysis Buffer) was added and the mixture was vortexed. Samples were pulsed and incubated at 56 °C for 10 minutes. To each tube, 500 µl of ethanol was added and vortexed and the mixture was transferred into a Qiagen mini spin column without wetting the rim and spun at 8000 rpm for 1 min. After each Qiagen mini spin column was placed in a new clean 2 ml collection tube and the tube containing the filtrate was discarded. The Qiagen mini spin columns were opened and 500 µl buffer AW1 was added and centrifuged at 8000 rpm for 1 minute. The tubes containing the filtrate were discarded and the Qiagen mini spin columns were placed in a new clean 2 ml collection tubes and 500 µl buffer AW2 was added and centrifuged at 14,000 rpm for 3 minutes. A second spin for 1 minute was applied in a

new clean 2 ml collection tube at full speed. The Qiagen mini spin columns each were placed in a clean 1.5 ml micro-centrifuge. The collection tube containing the filtrate was discarded. The Qiagen mini spin column was carefully opened and a volume of 100 µl buffer AE was added. The resulting solution was incubated for 15 minutes at 24^0C. It was then centrifuged at 8000 rpm for 1 minute. The eluted gDNA was quantified using NanoDrop 2000 (Thermo Scientific). Confirmation of the DNA extraction was conducted using 1% agarose gel containing 4 µl of Ethidium bromide for a gel electrophoresis at 100 volts.

3.11.2. Primer design process for the *EEFSEC, AGTR2, WNT4, ADCY5* SNPS

The flanking sequence of the variant positions for each gene was downloaded in the rtf format from ensemble.org and opened in Microsoft word. The sequence was copied into the primer quest tool in Integrated DNA Technologies (IDT) (idtdna.com) to generate primers. Sets of forward and reverse primers with sequence flanking the variants were selected and verified for percentage GC, GC clamp, self-annealing, hairpin formation and melting temperatures (Tm) to assess suitability of each of the primers for polymerase chain reaction (PCR) using the Sequence manipulation suite (https://www.bioinformatics.org/sms2/pcr_primer_stats.html). A hetero-Dimer analysis for PCR suitability was conducted using OligoAnalyzer tool (https://www.idtdna.com). Primer pairs with the maximum base pair Delta G of less than 9 kcal/mol were selected. The primer pairs were further assessed for specificity using UCSC In-Silico PCR tool of the UCSC Genome Browser (https://genome.ucsc.edu/cgi-bin/hgPcr). The forward and reverse primers used for the PCR and their corresponding genes and SNPs investigated are shown in **Table 3.1**

Table 3.1: A list of genes, SNPs and primer utilised for PCR and restriction digestion

Gene	Locus an SNP[π]	Primer orientation	Primer sequence (5′ - 3′)	Reference
SERPINH1	-656 C/T	Forward	*CCACTGTCGCCCAGATTATTTA*	(Wang et al., 2006)
		Reverse	*CAGTGCCCTTCTCCATACTTGT*	
MMP-1	-1607 G/GG	Forward	*TGCTGAGAATGTCTTCCCATT*	(Dunleavey et al., 2000)
		Reverse	*TCTTGGATTGATTTGAGATAAGTGAAATC*	
EEFSEC	rs2955117 (G/A)	Forward	*GGCTTGCCCTTGAGAACAAAAC*	
		Reverse	*GCTGTGGCTATGATTTCCTTCTG*	
AGTR2	rs5950491 (C/A)	Forward	*CTGGACCGTCACAGATGCT*	
		Reverse	*CTCAACCTAAATGGAAACTGG*	
WNT4	*rs56318008* (C/T)	Forward	*GAATCCGAAACCTCGCTTCT*	
		Reverse	*GCCCATTCCATTCCATTCATTT*	
ADCY5	*rs9861425* (A/C)	Forward	*CTCTCGACTCAGGTCTGCTTT*	
		Reverse	*GAGCAGGGGTCTTTCCTTATGA*	

3.11.3. Polymerase chain reaction process

A 20 µl volume of the extracted genomic DNA was each standardised into a concentration of 20 ng/µl for PCR. A PCR optimization of the primers were conducted to select appropriate denaturation and annealing temperatures for further PCR reactions. In the optimization process, PCR was carried out for 35 cycles in a total volume of 11 µl, containing 40 ng (2 µl of the 20 ng/µl) DNA, 5 pmol each primer, 2.5 µl QIAGEN Fast Cycling and 6 µl of Nuclease free water. A 5µl aliquot of the amplicons was mixed with 2 µl of loading buffer (New England Biolabs) and electrophoresed on a 2 % agarose gel for 1 hour. The gel was then scanned with the Amersham 6000 imager.

The total volume of 11 µl, containing 40 ng (2 µl of the 20 ng/µl) DNA, 5 pmol each primer, 2.5 µl QIAGEN Fast Cycling and 6 µl of nuclease free water was used subsequently for the PCR reaction to genotype the SNPs. The PCR conditions for each of the genes is shown in **Table 3.2.**

Table 3.2: PCR conditions for sequence amplification

GENE and SNP	PCR Conditions										
	Initial denaturation		Cycles	Denaturation		Annealing		Extension		Final Extension	
	Temp. (^{0}C)	Time (min)		Temp. (^{0}C)	Time (sec)	Temp. (^{0}C)	Time (sec)	Temp. (^{0}C)	Time (sec)	Temp. (^{0}C)	Time (min)
SERPINH1 - 656 C/T	94	5	35	94	45	60	45	72	60	72	10
MMP-1 -1607 G/GG	95	1	35	95	30	55	30	72	30	72	10
EEFSEC rs2955117 (G/A)	94	5	35	94	45	57.6	45	72	60	72	10
AGTR2 rs5950491 (C/A)	95	5	35	94	45	59.6	45	72	60	72	10
WNT4 rs56318008 (C/T)	94	5	40	94	45	56.7	45	72	60	72	10
ADCY5 rs9861425 (A/C)	94	5	35	94	45	52.7	30	72	60	72	10

3.11.4. Restriction endonuclease digestion

Aliquots of PCR products were mixed with reaction mix containing the appropriate buffer (New England Biolabs) and restriction enzymes (New England Biolabs) incubated at temperature prescribed by the manufacturers as shown in **Tables 3.3** and **3.4.**

Table 3.3: Aliquots of reagents for RFLP assay

GENE and SNP	Volume of PCR product (µl)	Endonuclease	Volume of endonuclease (µl)	Type of buffer	Volume of buffer (µl)	Volume of nuclease free water (µl)
SERPINH1 - 656 C/T	6	ApaL1	0.3	Rcut smart	1.8	2.95
MMP-1 -1607 G/GG	10	XmnI	0.3	Rcut smart	1.2	2.5
EEFSEC rs2955117 (G/A)	10	NIaIII	0.3	Rcut smart	1.2	2.5
AGTR2 rs5950491 (C/A)	10	M1uCI, TfiI, SspI	0.3	Rcut smart	1.2	2.5
WNT4 rs56318008 (C/T)	6	HgaI	0.3	r1.1	1.2	2.5
ADCY5 rs9861425 (A/C)	6	BtsI-v2	0.3	Rcut smart	1.2	2.5

Table 3.4: Reaction conditions for RFLP and products

GENE and SNP	Endo.*	Incubation conditions		Deactivation conditions		Fragments description	
		Temp. (^{0}C)	Duration (min)	Temp. (^{0}C)	Duration (min)		Length (bases)
SERPINH1 - 656 C/T	ApaL1	37	15	-	-	Length of amplicon	624
						Length of fragment with C allele	446, 178
						Length of fragment with T allele	624
MMP-1 -1607 G/GG	XmnI	37	180	65	20	Length of amplicon	118
						Length of fragment with GG allele	118
						Length of fragment with G allele	89, 29
EEFSEC rs2955117	NIaIII	37	120	65	20	Length of amplicon	157
						Length of fragment with A allele	58, 99
						Length of fragment with G allele	157
AGTR2 rs5950491	M1uCI	37	15	-	-	Length of amplicon	262
						Length of fragment with A allele	205, 57
						Length of fragment with C allele	262
	TfiI	65	15	-	-	Length of fragment with G allele	204, 58
	SspI	37	15	65	20	Length of fragment with T allele	203, 59
WNT4 rs56318008	HgaI	37	60	65	20	Length of amplicon	304
						Length of fragment with C allele	114, 190
						Length of fragment with T allele	304
ADCY5 rs9861425	BtsI-v2	37	15	-	-	Length of amplicon	404
						Length of fragment with A allele	404
						Length of fragment with C allele	253, 151

Endo.* = endonuclease

3.11.5. Gel electrophoresis

Two microlitres aliquot of each of the digests was mixed with 3 microlitres loading buffer (New England Biolabs) and electrophoresed on agarose gel with ethidium bromide for a duration at voltages as shown in **Table 3.5**. The gels were then scanned with Amersham 6000 imager.

Table 3.5: Conditions for gel electrophoresis used for six polymorphisms

GENE and SNP	Volume of digest (µl)	Volume of loading buffer (µl)	Percentage agarose (g/100 ml)	Volume of ethidium bromide (µl)	Duration (min)	Voltage (Volts)
SERPINH1 - 656 C/T	5	2	4	4	45	100
MMP-1 -1607 G/GG	10	3	6	4	90	120
EEFSEC rs2955117 (G/A)	5	2	5	4	60	100
AGTR2 rs5950491 (C/A)	5	2	4	4	60	100
WNT4 rs56318008 (C/T)	5	2	3	4	45	100
ADCY5 rs9861425 (A/C)	5	2	3	4	30	100

3.11.6. RNA extraction

The extraction was conducted using an optimised formalin-fixed paraffin embedded kit (Zymo Research). About 25 mg of the RNA later (Qiagen) fixed placental tissue was microdissectioned and added to 1000 µl of PBS, centrifuged at 16000g and the supernatant discarded. The tissue was digested with 95 µl DNase/RNase-free H_2O, 95 µl 2x digestion buffer, 10 µl proteinase K and incubated at 55°C for 4 hours. RNA lysis buffer (600 µl) was added to the tissue, mixed

thoroughly, centrifuged 16000g for 1 minute and debris removed. The supernatant was transferred into nuclease free tube and ethanol (95 – 100%) was added in the ration 1:1. The sample mixture was then transferred into a Zymo-Spin™ in a collection tube, centrifuged at 10000g for 30 seconds and the flow-through was discarded. This was followed by DNase treatment where 400 µl of RNA wash buffer was added to the column and centrifuged at 10000g for 30 seconds. The flow-through was discarded and 80 µl of DNase reaction mix (5 µl of DNase + 75 µl of DNA digestion buffer) was added. RNA prep buffer (400 µl) was added to the column and centrifuged at 10000 g for 30 seconds and the flow-through discarded. RNA wash buffer (700 µl) was added to the column, centrifuged at 10000g for 30 seconds and the flow-through discarded. RNA wash buffer (400 µl) was added to the column, centrifuged at 10000g for 60 seconds and the flow-through discarded. The column was then transferred to a nuclease-free tube. DNase/RNase-free water (50 µl) was directly added to the column matrix and centrifuged at 16000g for 30 seconds to elute the RNA. The concentration of the RNA was determined using nanodrop and immediately frozen at -80°C.

3.11.7. Primer design process and conditions for RT-qPCR

The primers for *COLLAGEN, TYPE 4, ALPHA 1 (COL4A1); COLLAGEN, TYPE 4, ALPHA 2 (COL4A2); COLLAGEN, TYPE 4, ALPHA 3 (COL4A3); PROLYL 4-HYDROXYLASE (P4H)* and *SERPINH1* mRNA were designed based on the accession number of each gene obtained from NCBI and the NCBI primer BLAST software. Primers for mature mRNAs were designed to span the exon–exon junction. Sets of forward and reverse primers were verified for percentage GC, GC clamp, self-annealing, hairpin formation and melting temperatures (Tm) to assess suitability of each of the primers for PCR using the Sequence manipulation suite

(Bioinformatics.org). The primer pairs were further assessed for specificity using UCSC In-Silico PCR tool of the UCSC Genome Browser. The forward and reverse primers and their corresponding genes investigated is shown in **Table 3.6**.

Table 3.6: A list of genes and primers used for RT-qPCR

Name of gene	Primer orientation	Primer sequence (5' to 3' direction)	Reference
COL4A1	Forward	GACGGTGCGTAGCGCTGGAAGT	
	Reverse	GTCCCTTCACTCCATGGCAGTCACA	
COL4A2	Forward	GGTGATGTCTGCTACTATGCCAGC	
	Reverse	GCTGATGTGTGTGCGGATGAG	
COL4A3	Forward	GTGCTTCTGTGACGGGGCCAAA	
	Reverse	CCTTCAGGACCTGTGAATCCTTTCTG	
P4H	Forward	GATCTGGTGACTTCTCTGAAAGA	
	Reverse	CCAACCTGATCTTCATCATTAGGA	
SERPINH1	Forward	AACGCCATGTTCTTCAAGCCACACT	(Wang *et al.,* 2006)
	Reverse	TAGTTGTAGAGGCCTGTCCGGTGCAT	
*GAPDH**	Forward	GTATCGTGGAAGGACTCATGACCA	
	Reverse	TAGAGGCAGGGATGATGTTCTGGA	

*GAPDH** = internal control

3.11.8. Quantitative polymerase chain reaction

The reaction was done using the Luna One-Step RT-qPCR kit (New England Bioloabs). An aliquot of each of the extracted RNA was standardised to obtain 20 ng/µl. A 10 µl reaction mix per each sample was prepared as illustrated in the **Table 3.7.** The conditions for the RT-qPCR transcript amplifications are exhibited in **Table 3.8.**

The RT-qPCR reaction focused on the expression of the *SERPINH1* based on the - 656 C/T SNP. The expression levels of *COL4A1, COL4A2, COL4A3* and *P4H* which are engaged in collagen synthesis upstream of the *SERPINH1* gene in the pathway were also assessed to determine whether there is any reduced-expression that could impact downstream *SERPINH1* activities. Additionally, samples were grouped based into Controls with *SERPINH1* - 656 C/T (n = 5), SPTBs with *SERPINH1* - 656 C/T (n = 5), Controls with *SERPINH1* - 656 C/C (n = 5) and SPTBs with *SERPINH1* - 656 C/C (n = 5). Only SPTBs, (n = 2) had *SERPINH1* - 656 T/T in this study. The RT-qPCR was conducted with *GAPDH* as the internal control. Each of the samples were run in triplicate.

Table 3.7: Aliquots of reagents for RT-qPCR assay

Component	Reaction volume (µl)
Master mix [Luna One-Step RT-qPCR kit (New England Bioloabs)]	5
Enzyme mix [Luna One-Step RT-qPCR kit (New England Bioloabs)]	0.5
Forward primer	0.4
Reverse primer	0.4
Template RNA	2.0
Nuclease-free water	1.7

Table 3.8: RT-qPCR conditions for transcripts amplification

Cycle step	Temperature (°C)	Time seconds	Cycle
Reverse transcription	55	600	1
Initial denaturation	95	60	1
Denaturation	95	10	40
Annealing	57	30	
Extension	60	60	
Melt curve	95	15	1
	60	60	
	95	60	

3.12. Statistical analysis

All data generated was stored in Excel spreadsheet and exported to R for clean-up and analysis. Pearson's Chi square (χ^2) test, ANOVA was used to compare demographic, allele, and genotype distributions between the cases (SPTBs) and the control groups (term mothers).

Allele frequencies were calculated by using the counts of the reference and alternate alleles observed across all genotyped individuals and dividing each count by the total number of alleles in the sample (twice the total number of individuals genotyped) (Griffiths *et al.,* 2015; Hartl & Clark, 2007) as shown in equations 1 and 2 below.

$$f(M) = \frac{2n[MM] + n[MS]}{2n_{total}} \quad \ldots\ldots\ldots 1$$

$$f(S) = \frac{2n[SS] + n[MS]}{2n_{total}} \quad \ldots\ldots\ldots 2$$

$$f(M) + f(S) = 1 \quad \ldots\ldots\ldots 3$$

M = reference allele, S = alternate allele, n = number of people genotyped
n_{total} = total number of people genotyped

Genotype frequencies were calculated by dividing the number of individuals with each genotype (homozygous genotype (reference alleles), heterozygous genotype and homozygous genotype (alternate alleles) by the total number of genotyped individuals (Griffiths *et al.,* 2015; Hartl & Clark, 2007) as shown in equations below.

$$N_{total} = n[MM] + n[MS] + n[SS] \ldots\ldots 1$$

$$f[MM] = \frac{n[MM]}{n_{total}} \quad \ldots\ldots\ldots 2$$

$$f[MS] = \frac{n[MS]}{n_{total}} \quad \ldots\ldots\ldots 3$$

$$f[SS] = \frac{n[SS]}{n_{total}} \quad \ldots\ldots\ldots 4$$

$$f[MM] + f[MS] + f[SS] = 1 \ldots\ldots 5$$

For quality control purposes to authenticate the allele frequencies calculated earlier, allele frequencies were recalculated using the equations below after the genotype frequencies had been calculated.

$$f[M] = f[MM] + \frac{1}{2} f[MS] \quad \ldots\ldots\ldots 1$$

$$f[S] = f[SS] + \frac{1}{2} f[MS] \quad \ldots\ldots\ldots 2$$

Relative fold changes in the RT-qPCR analysis were estimated using the ΔΔCt (Comparative Ct) and GABGH as the internal control (Wang *et al.,* 2006). The Ct values homozygous CC of the controls (Term births) was used as the control for *SERPINH1*(-656 C >T) expression analysis.

The gestational duration which was recorded in days was treated as a continuous trait and a quantitative trait while the term versus SPTB was considered a binary outcome. Also, univariate and multivariable logistic regression analyses were used to test for association between genetic loci and the risk of SPTB. Linear regression analyses were used to test for association between genetic loci and gestational duration. Variables that were not normally distributed were compared using the non-parametric Mann-Whitney U test. A *p-value* < 0.05 was considered statistically significant.

CHAPTER FOUR

4.0. RESULTS

4.1. Ethnic distribution and SPTB sub-type among participants

Overall, 604 participants consented to participate in the study and were enrolled. Participants were distributed across 29 ethnic groups. Of the 604 participants, the Akan ethnic group was the largest (n=228) comprising 103 mothers with SPTB and 125 with term birth). This was closely followed by women of Dagomba extraction (n =192), consisting of 90 mothers with SPTB and 102 with term birth. The number of participants with SPTB exceeded those with term birth among participants from Ewe, Fulani, Gonja and the Wala ethnic groups. Several other ethnic groups were represented in the study but with relatively fewer numbers, about 3 participants from these ethnic group (**Figure 4.1**, **panel A)** or **Table AI** of **Appendix A**. In terms of SPTB type, a high proportion (61%) of cases had (SPTL) compared to almost 39% of cases that had (PPROM) (**Figure 4.1**, **panel B**).

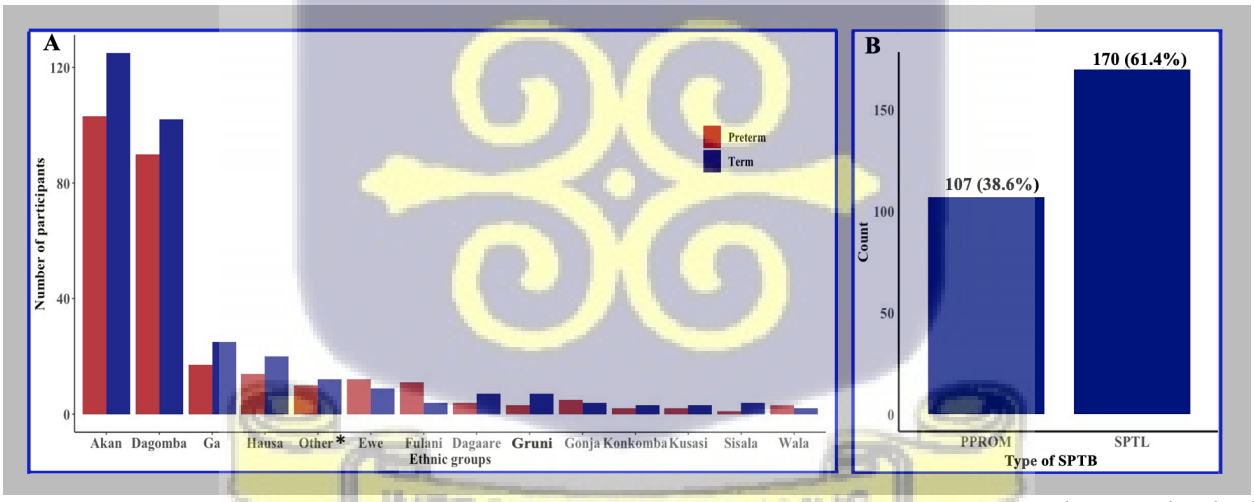


Figure. 4.1 Distribution of participants by ethnic group and SPTB subtypes. *About a third of the participants were from the Akan ethnic group. The "Other*" included a large group of ethnicities (Banda, Barsari, Bimoba, Builsa, Dangbe, Grusi, Guruma, Kotokoli, Krobo, Lobi, Mamprusi, Mosi, Mole, Sefwi, Wangara and Zabrama) which had a maximum of three participants each (panel A). About 6 out of 10 participants with SPTB had SPTL*

4.2. Demographic and clinical characteristics of participants

The age of SPTB cases ranged from 12 – 44 years whilst age of controls (women with term births) was 16 – 46 years. The mean age of mothers with SPTB was 27.0 (± 6.73SD) and that of controls was 28.0 (± 6.27SD), and difference was statistically significant ($p < 0.004$). Likewise, Hb at booking ranged from 7.6 – 14.3 g/dl for SPTB cases whilst Hb at booking of mothers with term births ranged from 7.7 – 13.9 g/dl. However, the mean Hb at booking for mothers with SPTB was 10.94 (± 0.93SD) and that of mothers with term birth was 11.13 (± 0.93SD), and difference was statistically significant ($p = 0.01$). Further details may be reviewed in **Table 4.1**.

Furthermore, maternal age was grouped into five age categories (<20, 20-25, 26-30, 31-35, >35) to explore the relationship between age and the risk of SPTB. Chi-squared test showed a significant difference ($p = 0.013$) in age groups between SPTB (cases) and Term births (controls). Additionally, marital status, antenatal visits and parity were also significantly different between the two groups at $p = 0.001$, $p = 0.01$ and $p = 0.03$ respectively. Hb at booking and age at menarche, however showed suggestive significance ($p = 0.09$) between mothers with SPTB (cases) and Term birth. Other maternal characteristics such as ethnicity, height, highest level of education, previous pregnancy outcomes and maternal Hb at booking did not differ significantly between cases and controls ($p > 0.05$) considering the Welch T test. Further details may be explored on **Table 4.2**.

Table 4.1: Comparison of means between variables of mothers with SPTB and Term birth

Variables	SPTB (277)		Term birth (327)		p - value$^{\mu}$
	Mean (±SD)	Range	Mean (±SD)	Range	
Age (years)	27.08 ± 6.73	12 – 44	28.63 ± 6.27	16 – 46	0.004**
Height (cm)	158.9 ± 6.27	141 – 180	159.7 ± 6.20	140 – 181	0.12
Age at menarche (years)	13.4 ± 1.33	10 – 16	13.27 ± 1.27	10 – 17	0.10
Hb at booking (g/dl)	10.94 ± 1.00	7.6 – 14.3	11.13 ± 0.93	7.7 – 13.9	0.01*

μ = Welch's t test

Table 4.2: Demographic and clinical characteristics of study participants.

Indicator	SPTB (277) n(%)	Term (327) n (%)	Total (604) n(%)	χ^2	*p* - value
Age (Years)					
<20	43(15.52)	23(7.03)	66(10.93)	12.69	0.013*
20-25	72(25.99)	83(25.38)	155(22.02)		
26-30	77(27.80)	99(30.28)	176(25.50)		
31-35	50(18.05)	78(23.85)	128(24.34)		
>35	35(12.64)	44(13.46)	79(13.07)		
Ethnicity					
Akan	103(37.18)	125(38.23)	228(37.75)	10.19	0.678
Dagomba	90(32.97)	102(31.19)	192(31.95)		
Ga	17(6.14)	25(7.65)	42 (6.95)		
Hausa	14(5.05)	20(6.12)	34(5.63)		
Ewe	12(4.33)	9(2.75)	21(3.48)		
Other ethnic groups	41(14.80)	46(14.07)	87(14.40)		
Height (cm)					
<155	53(19.13)	57(17.43)	110(18.21)	1.62	0.655
155-159	95(34.30)	101(30.89)	196(32.45)		
160-165	77(27.80)	103(31.50)	180(29.80)		
>165	52(18.77)	66(20.18)	118(19.54		
Education					
No formal education	44(15.88)	38(11.62)	82(13.58)	3.26	0.35
Primary	52(18.77)	50(15.29)	91(16.89)		
Junior High School	72(25.27)	93(28.44)	165(27.32)		
Senior High School	67(24.19)	88(26.91)	155(25.66)		
Tertiary	42(15.11)	58(17.74)	100(16.56)		

* = p value< 0.05, statistically significant, Chi-squared test was used to determine level of significance

Table 4.2: Demographic and clinical characteristics of study participants *continued*

Indicator	SPTB (277) n(%)	Term (327) n (%)	Total (604) n(%)	χ^2	*p* - value
Parity					
Nulliparity[α]	89(32.13)	73(22.32)	162(26.82)	7.37	0.03*
Para (1-4)	184(66.43)	248(75.84)	432(71.52)		
Grand multiparity	4(1.44)	6(1.83)	10(1.66)		
Maternal Hb at booking (g/dl)					
Moderate (7.0 – 9.9)	22(7.94)	14(4.28)	36(5.96)	6.48	0.09
Mild (10 – 10.9)	104(37.55)	105(32.11)	209(34.60)		
Normal (11 – 13.5)	147(53.06)	202(61.77)	349(57.78)		
High (13.6 – 15.0)	4(1.44)	5(1.53)	9(1.49)		
Antenatal visits					
≤ 3	97(35.02)	75(22.94)	172(28.48)	16.09	0.001**
4 - 5	102(36.82)	115(35.17)	217(35.93)		
6 - 7	62(22.38)	104(31.80)	166(27.48)		
≥ 8	16(5.78)	33(10.09)	49(8.11)		
Marital status					
Not married	69 (24.91)	53 (16.21)	122(20.20)	6.52	0.01*
Married	208 (75.09)	274 (83.79)	482 (79.80)		
Previous pregnancy outcomes					
Miscarriage	33(11.91)	34(10.40)	87(14.40)	0.21	0.64
No miscarriage	244(88.09)	293(87.77)	497(82.28)		
Stillbirth	8(2.89)	6(1.83)	14(2.32)	0.34	0.56
No stillbirth	269(97.11)	321(98.17)	590(97.68)		
Age at menarche (years)					
Early menarche (< 12)	30(10.83)	21(6.42)	51(8.44)	4.71	0.09
Menarche (12 – 14)	208 (75.09)	248(75.84)	456(75.50)		
Late menarche (≥ 15)	39(14.08)	58(17.74)	97(16.06)		

[α] = no previous child birth history apart from current birth. * = p value< 0.05, statistically significant, Chi-squared test was used to determine level of significance

4.3. Association of demographic and clinical characteristics with SPTB

To further explore the significant differences observed in section 4.1, where age group, parity, maternal Hb, antenatal visit and marital status differed between cases (SPTB) and controls (Term), univariate logistic regression was performed. The results showed that compared to mothers 26-30 years, mothers <20 years had an increased risk of pre-term birth (cOR=2.40, 95% CI [1.35 – 4.37], p =0.003). The risk remained significant (aOR = 1.9, 95% CI [1.03 – 3.57], p = 0.039) even after adjusting for parity, marital status, antenatal visits and ethnicity. Interestingly, antennal visits less 4 compared to mothers who visited greater than or equal to 4 times had an increased risk of SPTB (cOR = 2.76, 95%CI [1.39 – 5.32], p = 0.001). This remained significant after adjusting for parity, maternal age and ethnicity confounders (aOR = 2.55, 95% CI [1.31 – 5.11], p = 0.007).

Nulliparity as compared to para (1-4) was associated with an increased risk of SPTB (cOR = 1.64, 95% CI [1.14 – 2.37, p = 0.007], but this did not reach statistical significance after adjusting for antenatal visits and maternal age and ethnicity (aOR = 1.33, 95% CI [0.84 – 2.11], p = 0.23). Likewise, mothers who had moderate anaemia compared to mothers with normal Hb concentration were at an increased risk of SPTB (cOR =2.16, 95% CI [1.08 – 4.45], p =0.03) but failed to reach statistical significance after controlling for antenatal visits and ethnicity (aOR = 1.85, 95% CI [0.91 – 3.87], p = 0.1). Similarly, women who were not married as compared to the married women were associated with an increased risk of SPTB (cOR = 1.71, 95% CI [1.15 – 2.57, p = 0.008], but this did not reach statistical significance after adjusting for maternal age, parity and ethnicity (OR = 1.48, 95% CI [0.97 – 2.27], p = 0.07). (see **Table 4.3** for more details).

Table 4.3: Association between maternal characteristics and preterm birth

Variable	SPTB Count	Term Count	$_{c}$OR	95% CI	p - value	$_{a}$OR	95% CI	p - value
Age group (years)$^{\alpha}$								
<20	43	23	2.40	1.35 - 4.37	0.003**	1.9	1.03 - 3.57	0.04*
20-25	72	83	1.12	0.72 - 4.38	0.62			
26-30	77	99	Ref	Ref				
31-35	50	78	0.82	0.52 - 1.31	0.41			
>35	35	44	1.02	0.60 - 1.74	0.93			
Parity$^{\beta}$								
Nulliparity	89	73	1.64	1.14 - 2.37	0.007**	1.33	0.84 - 2.11	0.23
Para (1-4)	184	248	Ref	Ref				
Grand-multiparity	4	6	0.90	0.23 - 3.19	0.87			
Hb status at booking$^{\chi}$								
Moderate (7.0 - 9.9)	22	14	2.16	1.08 - 4.45	0.03*	1.85	0.91 - 3.87	0.1
Mild (10 - 10.9)	104	105	1.35	0.96 - 1.90	0.08			
Normal (11 - 13.5)	147	202	Ref	Ref				
High (13.6 - 15.0)	4	5	1.10	0.27 - 4.22	0.89			
Antenatal visits$^{\delta}$								
≤ 3	97	75	2.67	1.39 - 5.32	0.001**	2.55	1.31 - 5.11	0.007**
4 - 5	102	115	1.83	0.96 - 2.59	0.07			
6 - 7	62	104	1.23	0.63 - 2.46	0.55			
≥ 8	16	33	Ref	Ref				
Age at menarche (years)								
(<12)	30	21	1.70	0.95 - 3.10	0.08			
(12 – 14)	208	248	Ref	Ref				
(≥ 15)	39	58	0.90	0.56 - 1.41	0.38			
Marital status$^{\varepsilon}$								
Not married	69	53	1.71	1.15 - 2.57	0.008**	1.48	0.97 - 2.27	0.07
Married	208	274	Ref	Ref				

OR- Odds Ratio; CI- Confidence Interval expressed as (lower limit – upper limit); $_{c}$**OR** - crude Odds Ratio; $_{a}$**OR** - adjusted Odds Ratio; *= p value < 0.05, shows statistical significance; **Ref.:** Reference category; $^{\alpha}$ - adjusted for parity, marital status, antenatal visits and ethnicity; $^{\beta}$ - adjusted for antenatal visits, maternal age and ethnicity; $^{\chi}$ - adjusted for antenatal visits and ethnicity; $^{\delta}$ - adjusted for parity maternal age, and ethnicity; $^{\varepsilon}$ - adjusted for maternal age, parity and ethnicity.

4.4. Maternal depression and SPTB

The association between depression and SPTB was explored using the CES-D scores, as shown in **Table 4.4.** The table illustrates the results of depression among participants, expressed as CES-D scores, between SPTB and term-birth women. The mean CES_D scores for women with SPTB was 17.24 and that for the term was 13.51. The increased CES_D score among women with SPTB increased their risk of SPTB ($_c$OR = 1.85, 95% CI [1.33 - 2.57], p = 0.0002). The risk remained significant after adjusting for marital status ($_a$OR = 1.79, 95% CI [1.28 - 2.49], p = 0.0005).

Table 4.4: The risk of association between CES_D score and SPTB

Variable	SPTB, n = 277	Term, n = 327	$_c$OR	95% CI	*p*-value	$_a$OR	95% CI	*p*-value
$\bar{x}$ CES_D score (± SD)	17.24 (±10.98)	13.51 (±7.71)	1.85	1.33 - 2.57	0.0002***	1.79	1.28 - 2.49	0.0005***

$\bar{x}$ = mean, CES_D score = Centre for Epidemiologic Studies Depression score. CI- Confidence Interval expressed as (lower limit – upper limit), OR = odds ratio, $_c$**OR** = crude odds ratio, $_a$**OR** = adjusted odds ratio. *= p value < 0.05, shows statistical significance. Adjusted for marital status.

4.5. Placental histology and SPTB

The study additionally examined placental lesions as markers of adverse intrauterine condition associated with SPTB using 320 placental tissues (SPTB = 150) and (Term birth = 170). Chorioamnionitis was detected in 16% (24/150) placental tissues from the SPTB group compared with compared with 9% (15/70) among the term group. However, the difference was not statistically significant (p = 0.07) based on the chi square test as illustrated in **panel A** of **Figure 4.2**. The distribution of chorioamnionitis in the placental tissues from the SPTB women was further assessed based on three gestational age (weeks) categories: < 28, 28 – 33 and 34 – 36. The proportion of placental tissue with chorioamnionitis was highest at 25% (3/12) among the < 28-week group, and lowest 10.3% (8/78) among placental tissues in the 34 – 36 weeks category, as shown in in **panel B** of **Figure 4.2**. Photomicrograph of haematoxylin-eosin-stained Term and SPTB placental tissues exhibiting chorioamnionitis have also been illustrated in **Figure 4.3**.

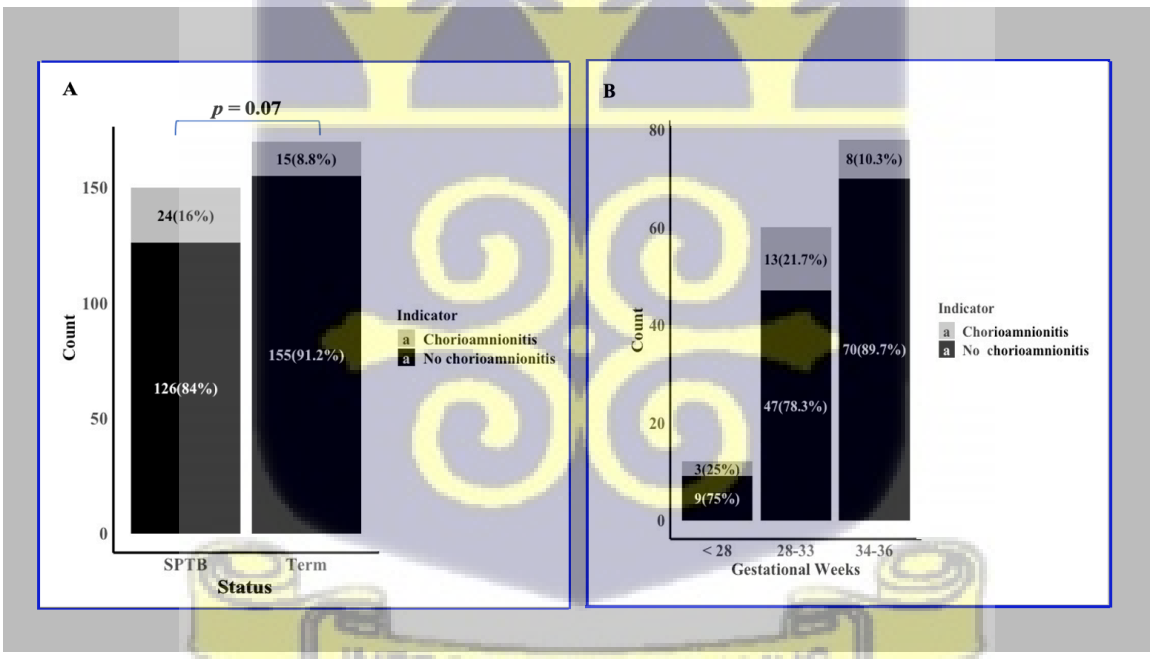


Figure 4.2: The distribution of chorioamnionitis among SPTB and term births placentae.
*Chorioamnionitis was detected in 16% (24/150) of SPTB placental tissues compared with compared with 9% (15/70) in term placental tissue. However, the difference was not statistically significant based on chi square test (**panel A**). A quarter of the number of placental tissues delivered than 28 weeks of gestation had chorioamnionitis and reduced to 10.3% (8/78) among placental tissues in the 34 – 36 weeks category (**panel B**).*

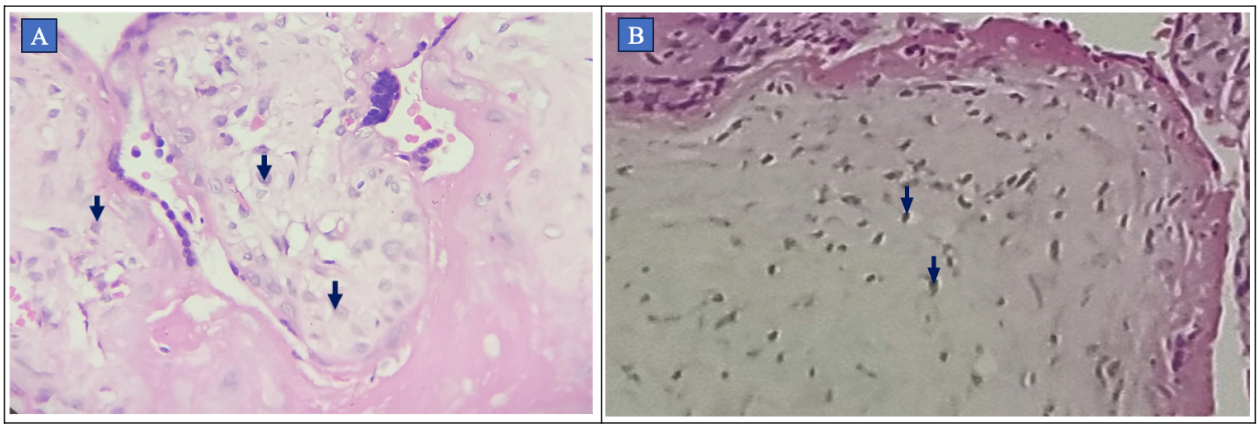


Figure 4.3: Photomicrograph of a SPTB and Term placentae with chorioamnionitis. ***Panel A*** *shows a photomicrograph of a 38-week term placenta with chorioamnionitis shown as neutrophils (black arrows); Haematoxylin and eosin stain; Magnification: 400x.* ***Panel B*** *shows a photomicrograph of a 28-week SPTB placenta with chorioamnionitis, neutrophils in black arrows. Haematoxylin and eosin stain; Magnification: 200x.*

The study further explored the placental lesions associated with maternal malperfusion. **Table 4.5** shows the distribution of placental lesions based on gestational ages categorised into weeks (< 28, 28 – 33 and 34 – 36) of the placentae. Placental lesions observed include: accelerated villous maturation, perivillous fibrin deposition, fibrinoid islands, distal villous hyperplasia, and villitis of unknown aetiology. All the lesions were identified in all the gestational age group except distal villous hyperplasia which was not identified in the < 28-week gestational age placental tissues. Perivillous fibrin deposition was the most identified lesion, with 25% (3/12), 26.67% (16/60) and 25.64 (20/78) in all the gestational age categories. Conversely, villitis of unknown aetiology and distal villous hyperplasia were the least observed lesions, with 10 placentae each from the 150 SPTB placental samples studied. Villitis of unknown aetiology noticed in 8.33% (1/12), 8.33% (5/60) and 5.13% (4/78) in the placental tissues from the < 28, 28 – 33 and 34 – 36-week gestational age categories respectively. Distal villous hyperplasia was however not identified in the < 28 gestation week placental tissues but was observed in the 28 – 33 and 34 – 36-week

gestational age categories at 10% (6/60) and 5.13% (4/78) respectively. Photomicrograph of haematoxylin-eosin-stained placental tissues displaying lesions have been illustrated in **Figure 4.4**.

Table 4.5: Placental lesion among SPTB based on gestational age

Lesion	SPTB (n = 150)		
	Gestational age (weeks) (n)		
	< 28 (12)	28-33 (60)	34-36 (78)
	n (%)	n (%)	n (%)
Accelerated villous maturation	1 (8.33)	9 (15.00)	6 (7.70)
Perivillous fibrin deposition	3 (25.00)	16 (26.67)	20 (25.64)
Fibrinoid islands	1 (8.33)	9 (15.00)	12 (15.38)
Distal villous hypoplasia	0 (0.00)	6 (10.00)	4 (5.13)
Villitis of unknown aetiology	1 (8.33)	5 (8.33)	4(5.13)

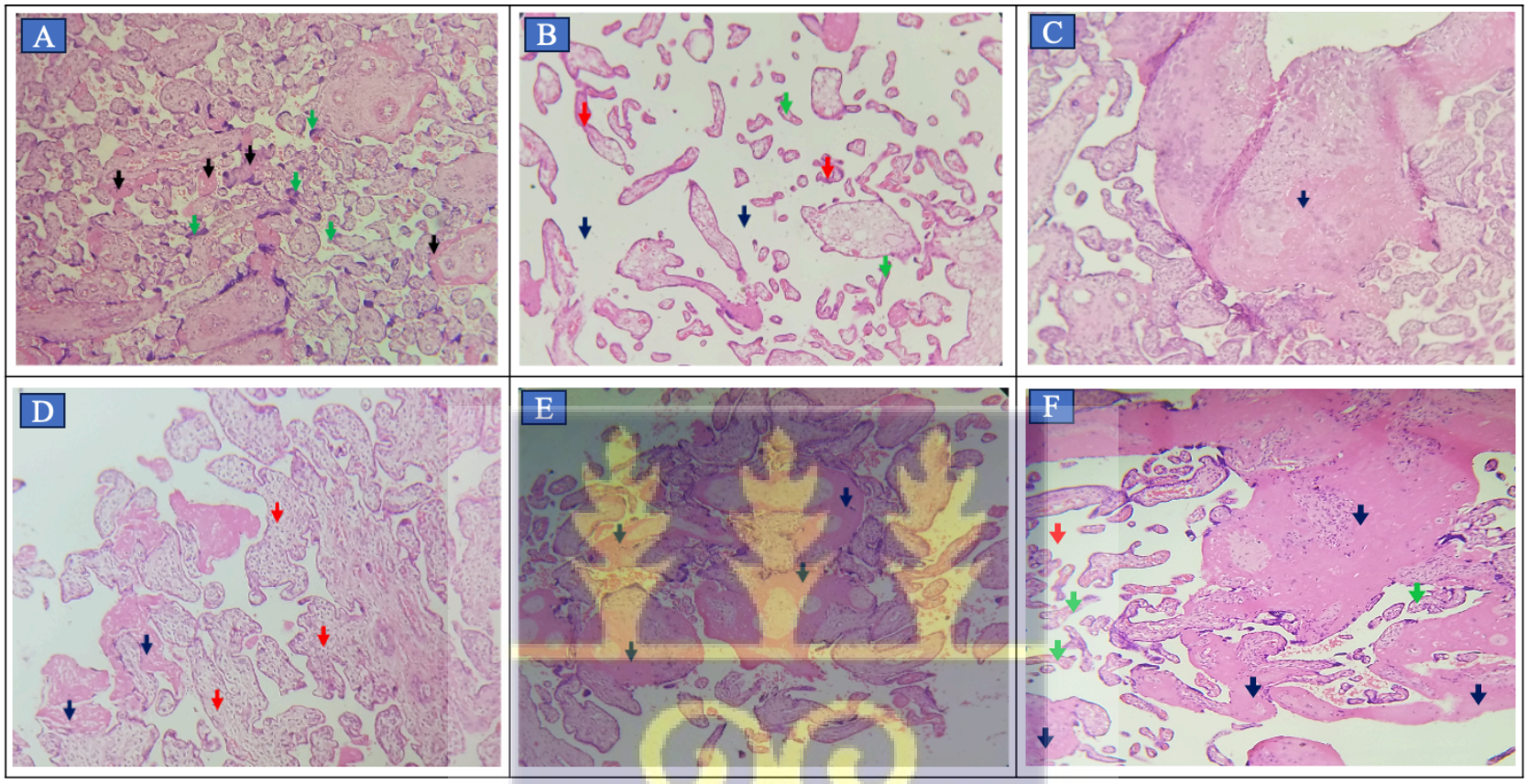


Figure 4.4: Photomicrographs of haematoxylin and eosin stained SPTB placental lesions. ***Panel A*** *shows a 26-week preterm placenta with increased pervillous fibrin deposition (black arrows) and increased syncytial knots (green arrows); Magnification: 100x.* ***Panel B*** *is a 35-week placental tissue with distal villous hypoplasia shown as increased intervillous space (black arrows), and increased syncitial knots (red arrows). It also shows accelerated villous maturation shown as small-for-gestational age, elongated villi (green arrows). Magnification: 100x.* ***Panel C*** *is a 32-week placental tissue showing fibrinoid island (black arrow). Magnification: 100x.* ***Panel D*** *is a 28-week placenta showing villi with villitis of unknown aetiology depicted as inflammatory cells (red arrows) in the villi. It also has and pervillous fibrin (black arrows). Magnification: 100x.* ***Panel E*** *is a 29-week placental tissue with perivillous fibrin (black arrow). Magnification: 100x.* ***Panel F*** *is a 34-week placental tissue with fibrinoid islands (black arrow); distal villous hypoplasia shown as sparce villi with large intervillous space (red arrow); accelerated villous maturation shown as elongated villi (green arrow). Magnification: 100x.*

4.6. Neonatal characteristics

The study focused on singleton births, as a result the total number of neonates assessed were 604 made up of 277 SPTB neonates and 327 term neonates, same as the number of mothers studied. When the SPTB neonates were grouped based on extreme preterm (< 28), very preterm (28 – 33 weeks) and late preterm (34 – 36) categories, late preterm neonates constituted 49.8% (138/277) while proportion of extremely preterm neonates was (7.9%) (22/277) as shown in **Figure 4.5** below.

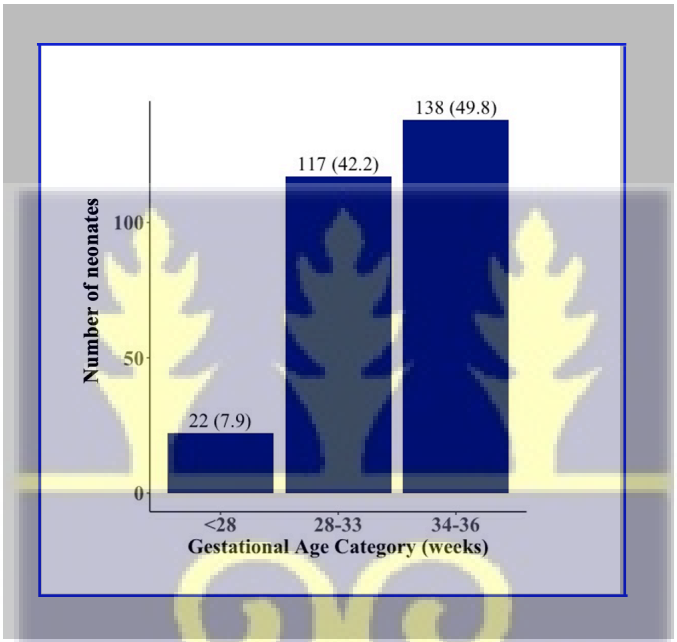


Figure 4.5: Distribution of gestational age categories of SPTB neonates. *The 34-36 weeks category composed 49.8% while <28 weeks made up 7.9%.*

To comprehend the impact of shortened duration due to SPTB on neonatal outcomes, neonatal anthropometric characteristics including: birth weight, length, head circumference as well as Apgar score (1st minute and 5th minute Apgar scores) were assessed. Obviously, the term neonates had significantly higher medians of the characteristics measured compared with the SPTB neonates ($p < 0.00001$) as shown in **Figures 4.6.** The median gestational ages for SPTB and term neonates were 33 and 39 weeks, respectively **Figures 4.6 panel A**. The gestational ages ranged from 24 to 36 weeks for SPTB and 37 to 42 for the term neonates. Regarding the birth weight, the median for the SPTB was 1.9 kg significantly lower than the 3.09 kg of the term neonate ($p <$

0.00001) as shown in **Figures 4.6 panel B**. The birth weight for SPTB ranged from 0.5 to 3.2 kg while that of the term neonates ranged from 2.2 to 4.31 kg. Invariably, the median head circumference for SPTB and term neonates were 29.8cm and 33.6 cm, respectively, significantly different from each other ($p < 0.00001$) as illustrated in **Figures 4.6 panel C**. The head circumference ranged from 21.7 cm to 35.9 cm among the SPTB neonates and 29.5 - 37.4 among the term neonates. Moreover, the SPTB neonates had a median birth length of 43.3 cm, significantly lower than 49.5 cm for the term neonates ($p < 0.00001$) as shown in **Figures 4.6 panel D**. The length also ranged from 29.2 - 52.6 cm and 41.8 - 55.6 cm among the SPTB and the term neonates respectively.

The first- and fifth-minute Apgar scores for the SPTB and term neonates was apparently dichotomous with the term neonates exhibiting better developmental attributes compared with SPTB neonates. The median first minute Apgar Score of 6 for the SPTB was significantly lower than the 8 for the term neonates ($p < 0.00001$) as shown in **Figures 4.6 panel E**, and ranging from 2 to 8 for SPTB and 6 to 9 cm for the term neonates. Likewise, the median fifth minute Apgar Scores for SPTB and term neonates as shown in **Figures 4.6 panel F** were 7 and 9 respectively, and significantly different ($p < 0.00001$). The fifth minute Apgar Score also ranged from 1 to 9 for SPTB and 7 to 9 cm for the term neonates (**Table AII of the appendix A**).

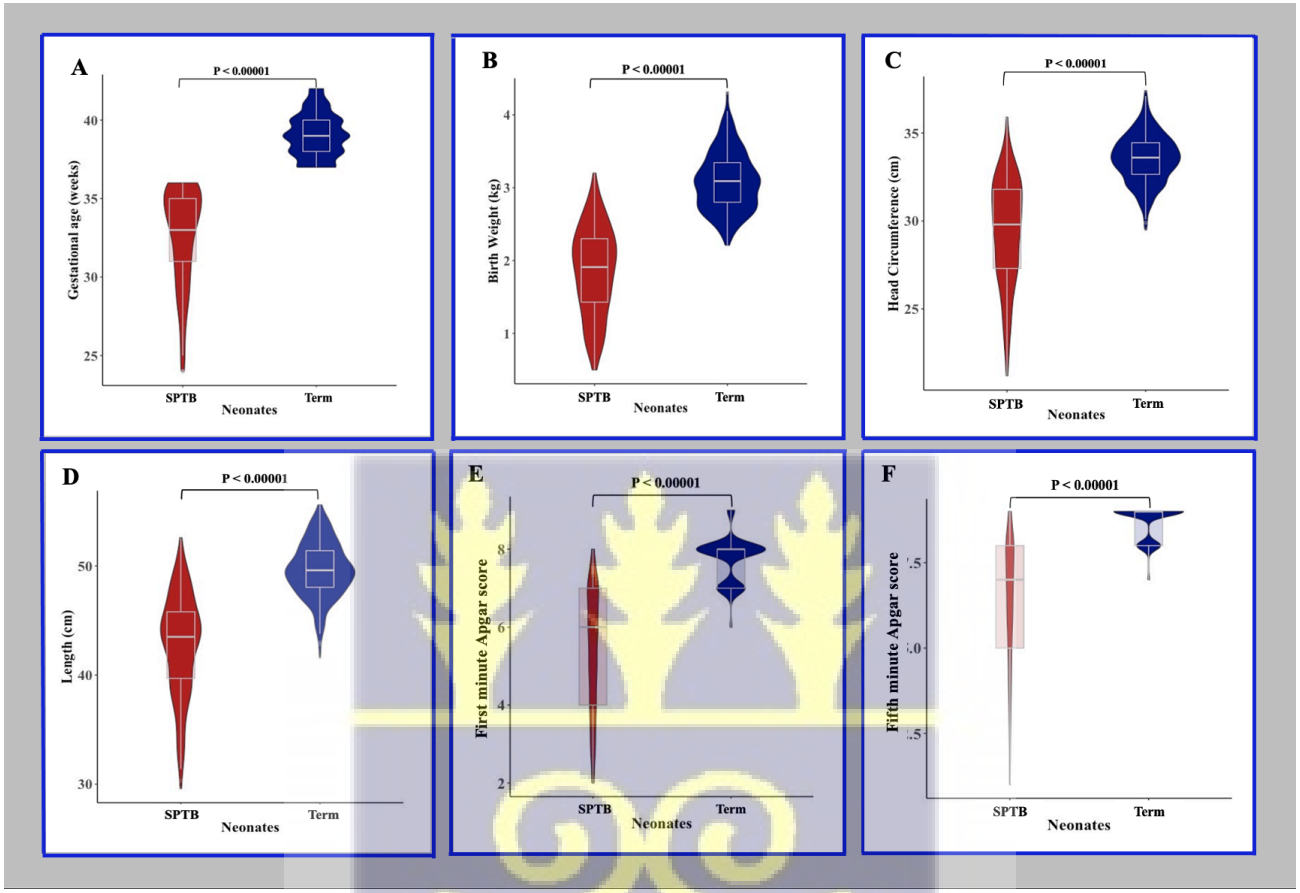


Figure 4.6: Characteristics of SPTB and term neonates. *The violin and box plot compares the medians of gestational ages* ***(panel A)****, birth weight* ***(panel B),*** *head circumference* ***(panel C),*** *length* ***(panel D),*** *first minute Apgar Score* ***(panel E)*** *and fifth minute Apgar Score* ***(panel F)*** *of SPTB and term neonates using the Mann-Whitney U test. The characteristics of the term neonates was significantly different from the SPTB neonates in all the measures assessed ($p < 0.00001$).*

The adverse outcome of the neonates was further investigated by assessing the distribution of the neonates' first- and fifth-minute Apgar scores categorised into 7 – 10 (reassuring or good to excellent health), 4 – 6 (fair or moderately abnormal), and 0 – 3 (low very low) Apgar scores. Notably, over 67% of neonates (407/604) had Apgar scores between 7 to 10 in the first minute. However, proportion of neonates in this Apgar score range increased to nearly 80% (479/604) by the 5th minute (**Figure 4.7**). Considering the neonates with 0 – 3 (very low) Apgar score, the proportion of the neonates however decreased from 7% (43/604) in the first minute to 3.5% (21/604) while in the fifth minute respectively (**Figure 4.7**).

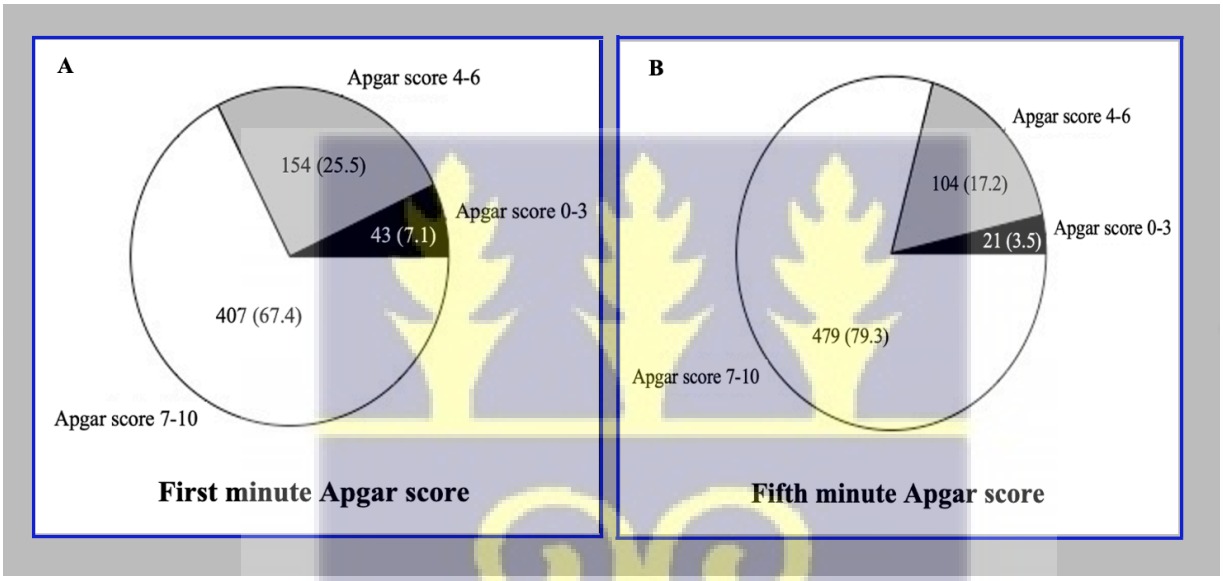


Figure 4.7: Distribution of the neonates' first- and fifth-minute Apgar scores *Panels A and B illustrates the proportions of neonates in the first- and fifth-minute Apgar score respectively categorised into 7 – 10 (reassuring or good to excellent health), 4 – 6 (fair or moderately abnormal), and 0 – 3 (low very low) outcomes.*

Additionally, based on the neonatal anthropometric indicators measured, a foetal growth analysis was carried out using scatter plots overlaid with smooth curves, harnessing the median birth weight, HC, and length, along with their corresponding completed gestational weeks at delivery. Coincidentally, the growth of all the three anthropometric indices peaked at the 41st week of gestation.

Difference in birth weight between males and females was not clearly visible between the 24th to the 29th weeks of gestation. However, a difference between the two genders became apparent from the 30th week till the 42nd week, with the males having superior weight-for-gestational age compared with the female counterparts. The median birth weight for the male neonates increased from 0.66 kg at the 24th gestational week and peaked at 3.31 kg at the 41st week, while that of the female increased from 0.70 kg at 24 weeks gestation to 3.20 kg at the 41st week **(Figure 4.8, panel A)** or **Tables AIII** and **AIV** of the **Appendix A.** A noticeable gender difference was however observed in the growth trajectory of the HC from the 25th week and 24th week for the length, with the males' curves lying above the females. The median HC for the male neonates increased from 22.25 cm at the 24th gestational week and peaked at 34.25 cm the 41st week. For the female neonates, their HC increased from 22.45 cm at the 24th gestational week and peaked at 33.80 cm at the 41st week **(Figure 4.8, panel B)** or **Tables AV** and **AVI** of the **Appendix A**. The birth length for the male neonates increased from 31.1 cm at the 24th gestational week and climaxed at 50.6 cm the 41st week. That of the female neonates increased from 31.4 cm at the 24th gestational week and also peaked at 50.1 cm at the 41st week **(Figure 4.8, panel C)** or **Tables AVII** or **AVIII** of the **Appendix A**.

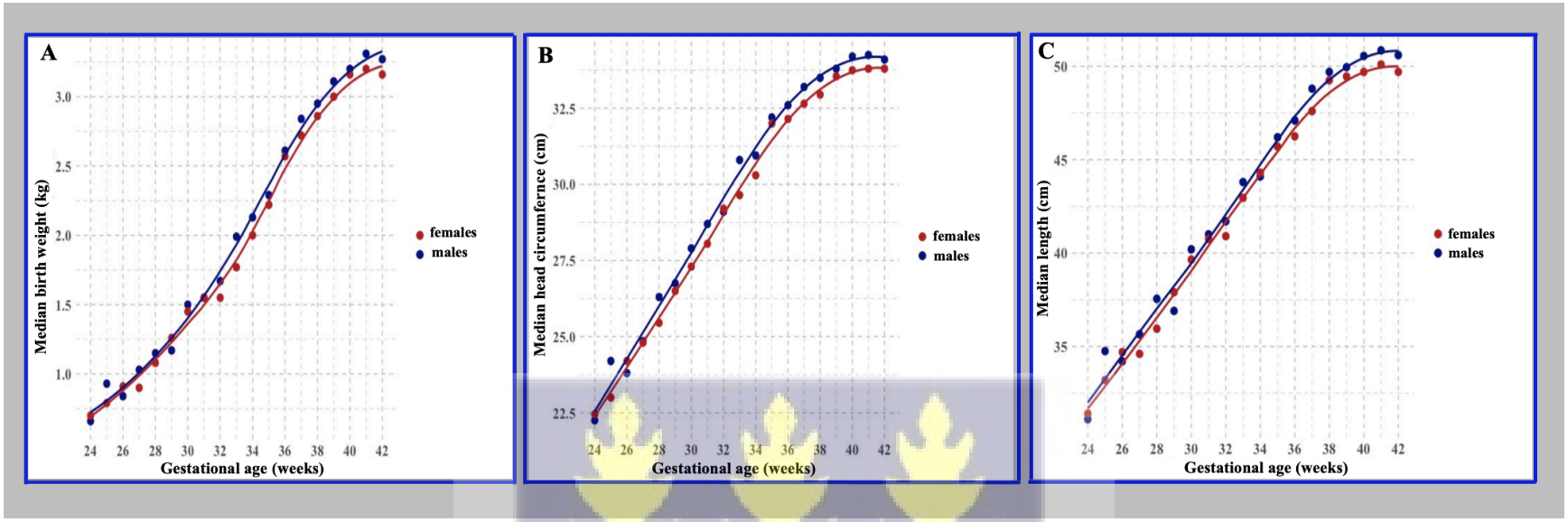


Figure 4.8: Neonatal anthropometric indicators. *The medians of All the three indicators, measured from 24 to 42 gestational weeks represented with scatter plot and smooth curve overlay. The median birth weight for the males rose from 0.66 kg at 24 weeks and peaked at 3.31 kg at 41 weeks. That of the females was 0.70 kg at 24 weeks but culminated at 3.20 kg at 41 weeks (**panel A**). The median head circumference for both the males and females were 22.25 cm and 22.45 cm respectively at 24 weeks but topped out at 34.25 cm and 33.80 cm respectively at 41 weeks (**panel B**). Invariably, the median lengths were 31.10 cm and 31.4 cm for males and females respectively at 24 weeks, and peaked at 50.58 cm and 50.10 cm for males and females respectively at 41 weeks (**panel C**).*

4.7. Candidate gene association with SPTB in Ghana

Persons that were successfully genotyped were 525 (86.9%) out of the 604 participants recruited. For the remaining 79 (13.1%), 63 consented to take part in the study without donating blood for DNA extraction, and the remaining 16 blood samples had unsuccessful RFLP results, possibly due to DNA degradation. Over half 280 (53.33%) of the successfully genotyped women had term births. Among those with SPTB, 62% were SPTL (**Figure 4.9**).

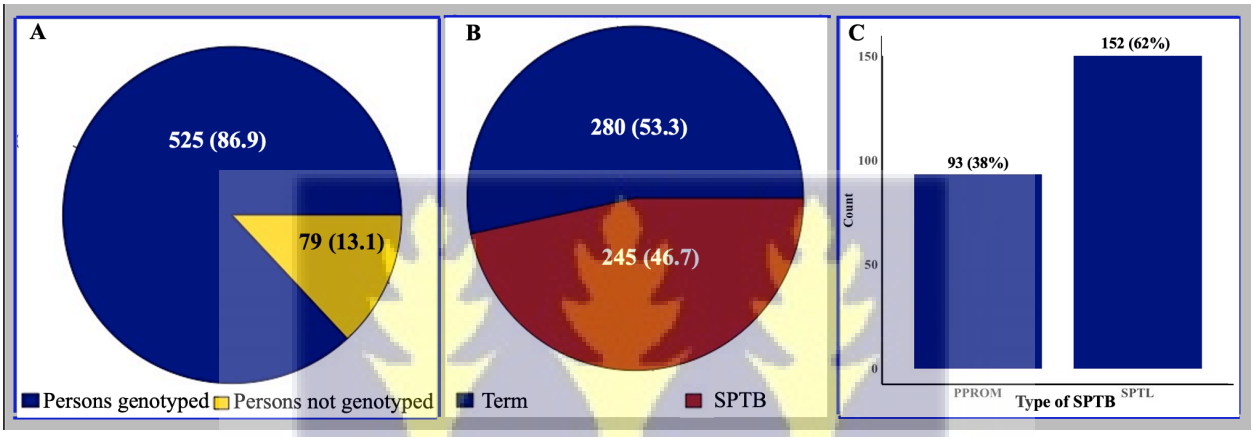


Figure 4.9: Distribution of participants successfully genotyped and birth outcomes. *Participants that were successfully genotyped were almost 87% (**panel A**), out of which over 53% had term births (**panel B**), and 38% of those with SPTB were PPROM (**panel C**).*

4.7.1. SNP genotyping using RFLP

A representative gel of the 525 samples used to determine genotypes for the candidate SNPs *ADCY5* (rs9861425-A/C), *SERPINH1*-656(C/T), *AGTR* (rs5950491), *WNT4* (rs56318008C/T), EEFSEC (rs2955117G/A)) and *MMP1* (-1607 1G/2G) investigated in each gene is shown in **Figures 4.10** to **4.17**

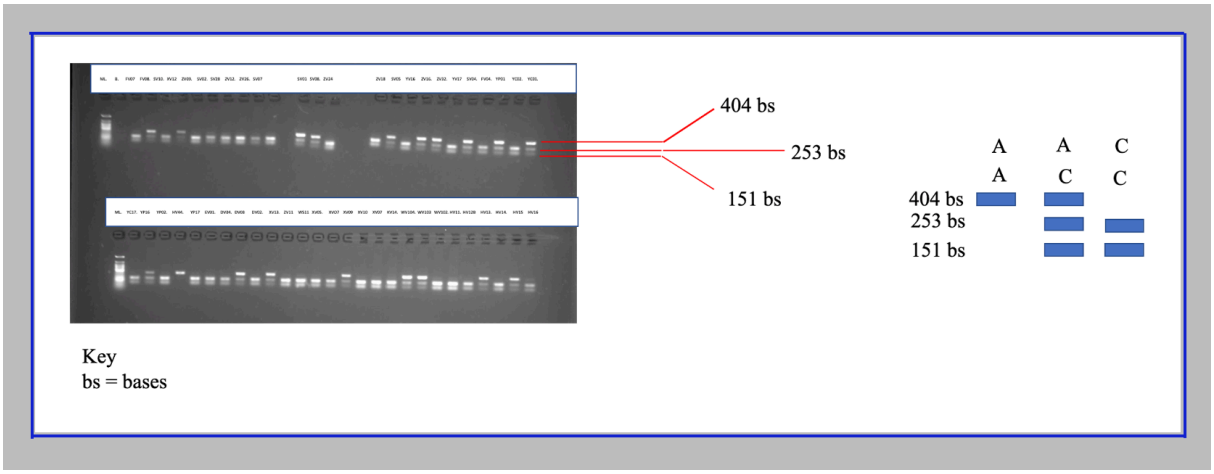


Figure 4.10: Gel image of restriction digestion of ADCY5 rs9861425 using BtsI-v2 enzyme*. The restriction digestion of a 404 base-pairs DNA amplicon containing the SNP (rs9861425) resulted in three fragments: 404bases, 253 bases and 151 bases. The blue rectangles on the right of the gel image illustrates three possible genotypes (AA, AC, CC) at the locus of the SNP. The AA exhibited a single band with 404 base pair (bp) while the AC genotype displayed three bands made up of: 404, 253 and 151 bases. The CC genotype showed bands with 253 and 151 bp.*

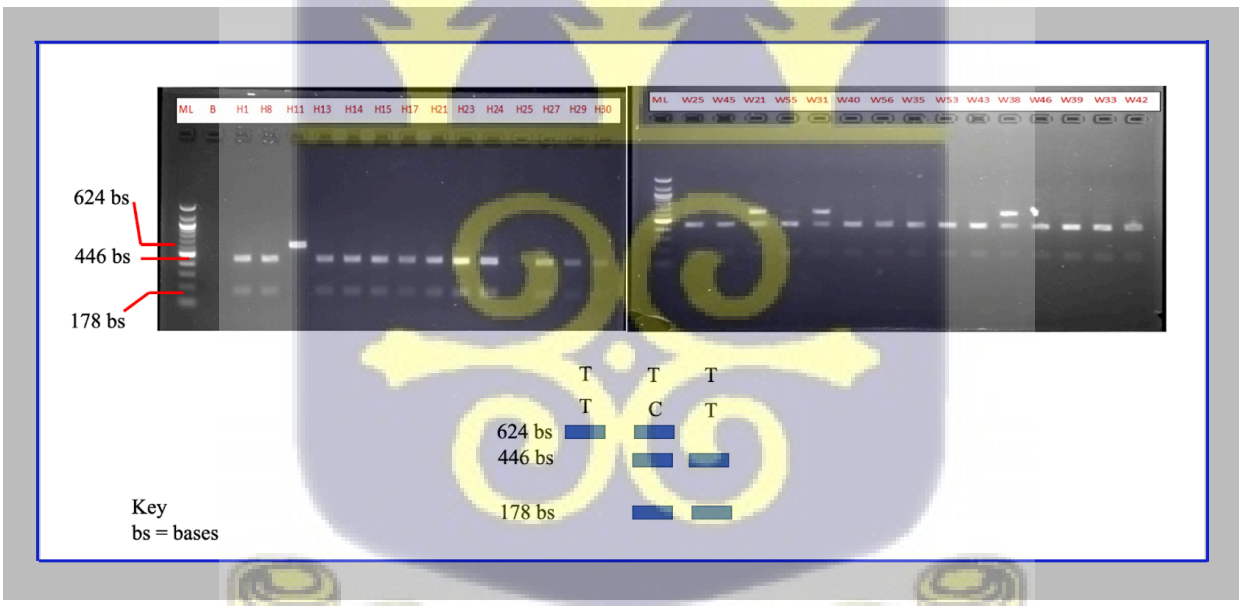


Figure 4.11: Gel image of restriction digestion of SERPINH1 -656 using ApaL1 enzyme. *The restriction digestion of a 624 base-pairs DNA amplicon containing the SERPINH1 -656 (C/T) resulted in three fragments: 624 bases, 446 bases and 178 bases. The blue rectangles below the gel image illustrates three possible genotypes (TT, CT, CC) at the locus of the SNP. The TT exhibited a single band with 624 bp while the CT genotype displayed three bands made up of: 624, 446 and 178 bases. The CC genotype showed bands with 446 and 178 bp.*

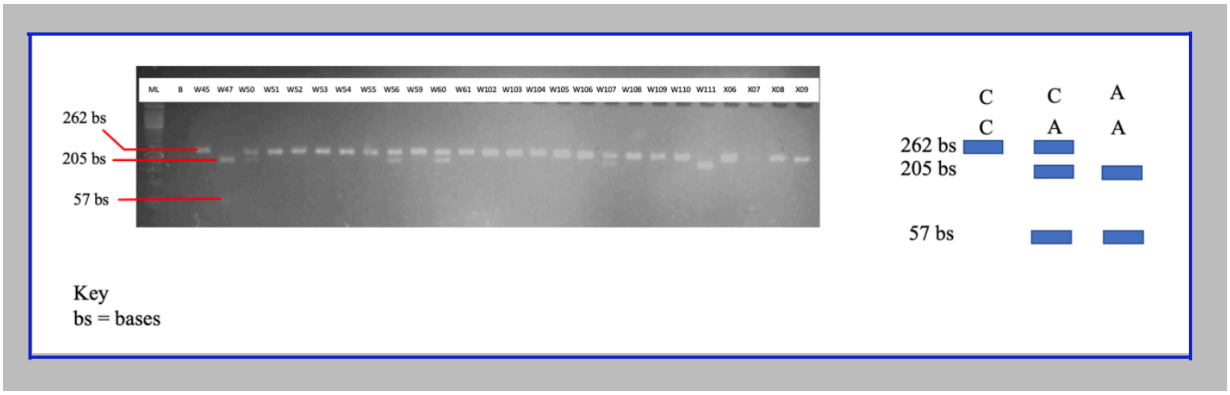


Figure 4.12: Gel image of restriction digestion of AGTR rs5950491 using M1uCI enzyme. *This restriction digestion of a 262 base-pairs DNA amplicon containing AGTR rs5950491 (C/A/G/T) resulted in three fragments: 262 bases, 205 bases and 57 bases. The blue rectangles on the right of the gel image illustrates three possible genotypes (CC, AC, AA) at the locus of the SNP. The CC exhibited a single band with 262 bp while the AC genotype displayed three bands was made up of: 624, 446 and 178 bases. The CC genotype showed bands with 205 and 57 bp*

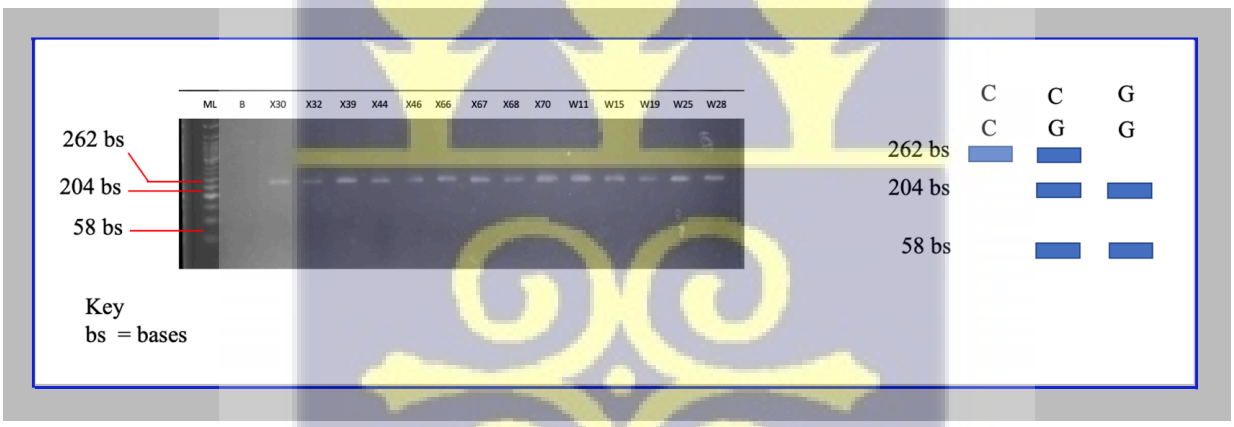


Figure 4.13: Gel image of restriction digestion of AGTR2 rs5950491 using TfiI enzyme. *The 262 base-pairs DNA amplicon containing AGTR2 rs5950491 (C/A/G/T) was not digested by the TfiI restriction enzyme. The blue rectangles on the right of the gel image illustrates three possible genotypes (CC, CG, GG) at the locus of the SNP if there had been restriction digestion by the TfiI restriction enzyme. The CC would have exhibited a single band with 262 bp while the CG genotype would have displayed three bands was made up of: 262, 204 and 58 bases. The GG genotype showed bands with 204 and 58 bp.*

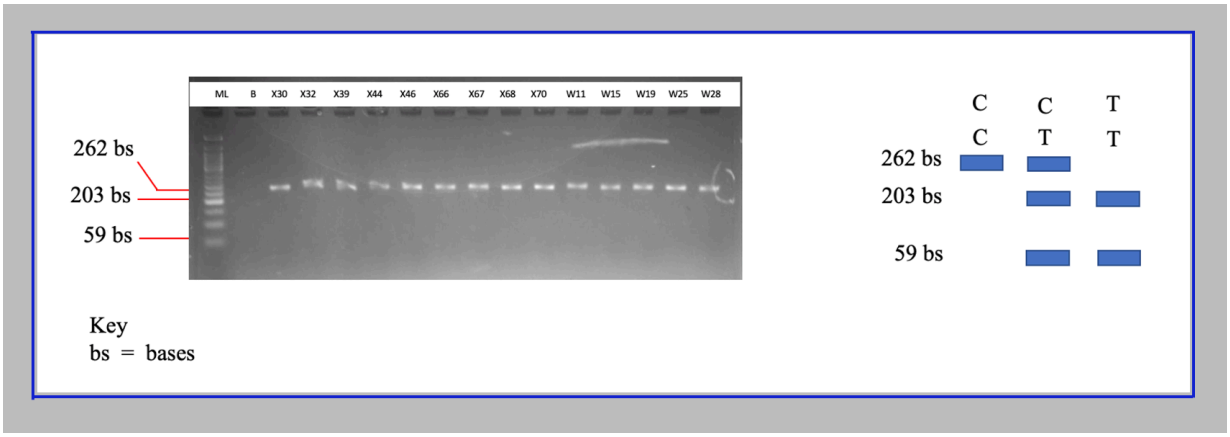


Figure 4.14: Gel image of restriction digestion of AGTR2 rs5950491 using SspI enzyme. *The 262 base-pairs DNA amplicon containing AGTR2 rs5950491 (C/A/G/T) was not digested by the SspI restriction enzyme. The blue rectangles on the right of the gel image illustrates three possible genotypes (CC, CT, TT) at the locus of the SNP if there had been restriction digestion by the SspI restriction enzyme. The CC would have exhibited a single band with 262 bp while the CT genotype would have displayed three bands was made up of: 262, 203 and 59 bases. The TT genotype showed bands with 203 and 59 bp.*

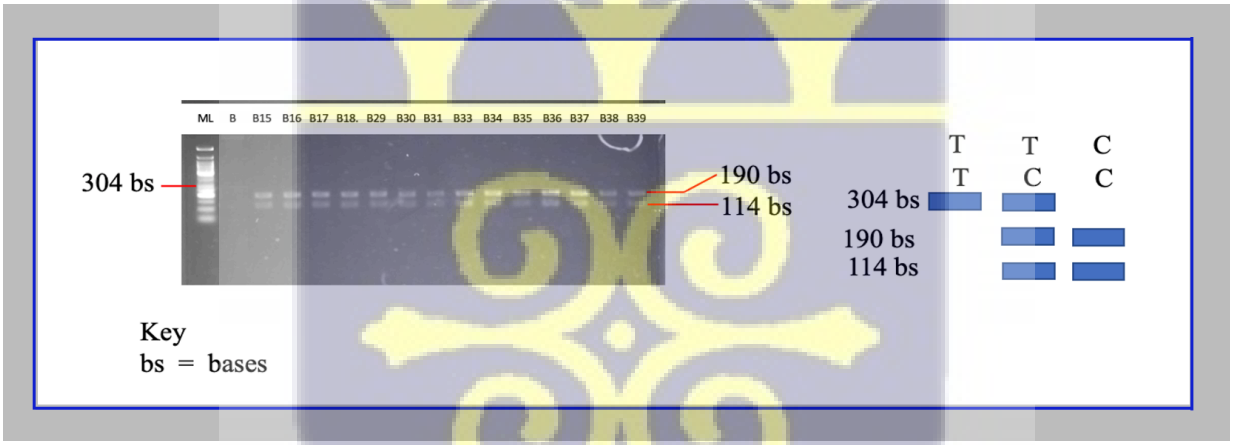


Figure 4.15: Gel image of restriction digestion of WNT4 rs56318008 using HgaI enzyme. *The 304 base-pairs DNA amplicon containing AGTR rs56318008 (C/T) was completely digested by the HgaI restriction enzyme. The blue rectangles on the right of the gel image illustrates three possible genotypes (TT, CT, CC) at the locus of the SNP based on restriction digestion by the HgaI restriction enzyme. The TT would have exhibited a single band with 262 bp while the CT genotype would have displayed three bands was made up of: 304, 190 and 114 bases. The CC genotype showed bands with 190 and 114 bp.*

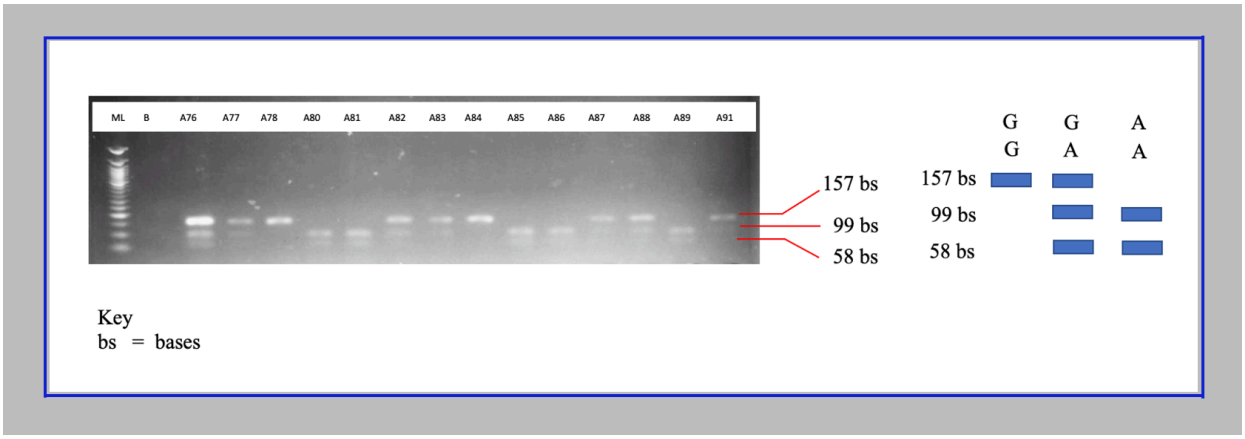


Figure 4.16: Gel image of restriction digestion of EEFSEC *rs2955117* ***using N1aIII enzyme.*** *The restriction digestion of a 157 base-pairs DNA amplicon containing the rs2955117 resulted in three fragments: 157 bases, 99 bases and 58 bases. The blue rectangles on the right of the gel image illustrates three possible genotypes (GG, AG, AA) at the locus of the SNP. The GG exhibited a single band with 157 bp while the AG genotype displayed three bands made up of: 157, 99 and 58 bases. The AA genotype showed bands with 99 and 58 bp.*

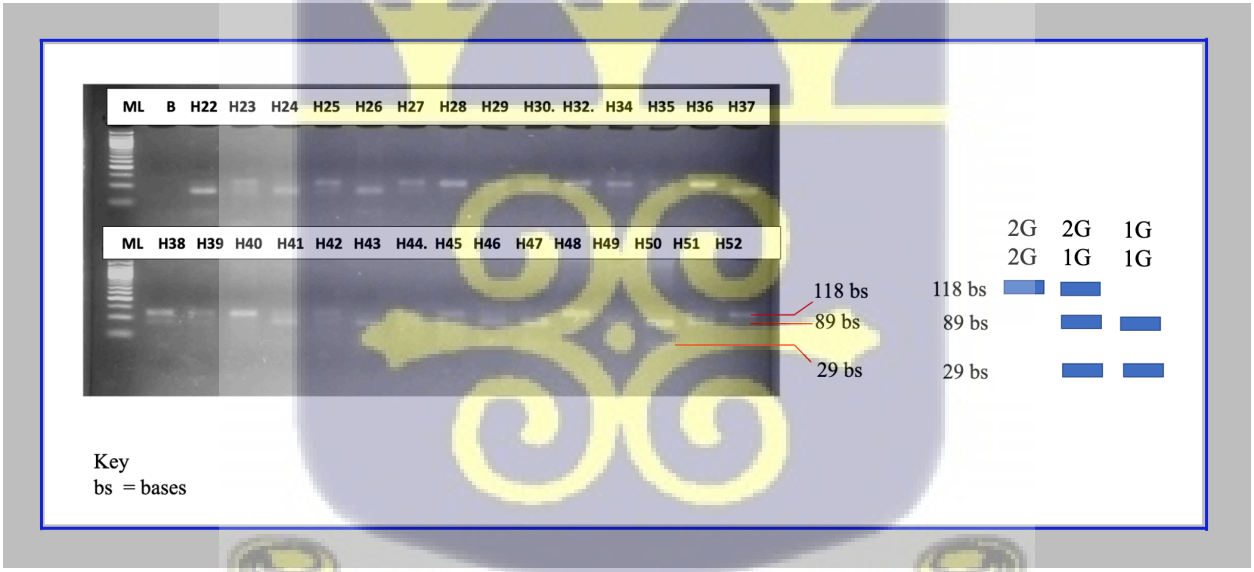


Figure 4.17: Gel image of restriction digestion of MMP1-1607 using XmnI enzyme. *The restriction digestion of a 118 base-pairs DNA amplicon containing the MMP1-1607 (1G/2G) resulted in three fragments: 118 bases, 89 bases and 29 bases. The blue rectangles on the right of the gel image illustrates three possible genotypes (2G2G, 2G1G, 1G1G) at the locus of the SNP. The 2G2G exhibited a single band with 157 bp while the 1G2G genotype displayed three bands made up of: 118, 89 and 29 bases. The 1G1G genotype showed bands with 89 and 29 bp.*

4.7.2. Allele frequency by SPTB and term birth

Table 4.6 shows the alleles detected in this study and their frequencies. Interestingly, the T allele of the *WNT4* rs56318008 (C/T) as well as the G and T alleles of the *AGTR2* rs5950491 (C/A/G/T) were not detected after restriction digest. Therefore, *WNT4* rs56318008 (C/T) was found to be monomorphic in this dataset and was not included any further analysis. Allele frequencies were comparable across the two groups, except for the T allele of *SERPINH1* (- 656 C/T) which was higher in the STPB group (0.12) compared to the Term birth (0.08).

Table 4.6: Allele frequencies of genotyped SNPs among Ghanaian women.

Locus	Allele	SPTB n (frequency)	Term Birth n (frequency)	Total n (frequency)
MMP-1 (-1607 1G/2G)	1G	250 (0.51)	303 (0.55)	553 (0.53)
	2G	240 (0.49)	257 (0.45)	497 (0.47)
EEFSEC rs2955117 (G/A)	G	298 (0.61)	334 (0.60)	632 (0.60)
	A	192 (0.39)	226 (0.40)	418 (0.40)
AGTR2 rs5950491 (C/A/G/T)	C	419 (0.86)	490 (0.88)	909 (0.87)
	A	71 (0.14)	70 (0.12)	141 (0.13)
	G	0 (0.00)	0 (0.00)	0 (0.00)
	T	0 (0.00)	0 (0.00)	0 (0.00)
ADCY5 *rs9861425* (A/C)	A	130 (0.27)	143 (0.26)	273 (0.26)
	C	360 (0.74)	417 (0.74)	777 (0.74)
SERPINH1 (- 656 C/T)	C	430 (0.88)	516 (0.92)	946 (0.90)
	T	60 (0.12)	44 (0.08)	104 (0.10)
WNT4 *rs56318008* (C/T)	C	490 (1.00)	560 (1.00)	1050 (1.00)
	T	0 (0.00)	0 (0.00)	0 (0.00)

4.7.3. Genetic loci association with gestational duration

A further analysis was conducted to using linear regression analysis to assess the association between the risk alleles of the five loci and gestational duration (**Table 4.7**). Each of the T allele in a particular locus was found to decrease gestational age by over 2 days (effect size -2.35, $p < 0.02$). The other risk alleles showed varying associations with gestational duration. However, their effects were not significant ($p < 0.05$).

Table 4.7: Risk alleles frequencies and their association with gestational duration

Loci	Alleles (A/B$^{\alpha}$)	Frequency	Effect size	p - value
ADCY5 rs9861425	A/C	0.74	-0.59	0.78
AGTR2 rs5950491	C/A	0.87	-3.03	0.22
EEFSEC rs2955117	G/A	0.40	0.25	0.89
MMP-1 (-1607 1G/2G)	1G/2G	0.47	-0.82	0.63
SERPINH1 (- 656 C/T)	C/T	0.10	-2.35	0.02

$^{\alpha}$ = B is the risk allele, $^{\beta}$ A positive value shows a longer gestational duration (Zhang *et al.,* 2017)

4.7.4. Allele and genotype association with SPTB

Univariate logistic regression analysis was undertaken to assess the association between each of the alleles of five polymorphic genes (**Table 4.8**) with SPTB. The T allele of *SERPINH1* - 656 C/T was found to increased risk of SPTB (OR = 1.63, 95%CI [1.08 – 2.46], p = 0.018). A chi square trend analysis demonstrated that genotype frequencies for the *SERPINH1* (- 656 C/T) were not comparable (**Table 4.9**). Genotype analysis based on the model of inheritance namely: the dominant, recessive, additive and the heterozygous models of inheritance assessed for these SNP markers, **Table 4.10** shows a statistically significant increased risk was observed with the

additive genetic model with an increased risk of SPTB (OR =1.77, 95%CI: [1.17 - 2.71], p < 0.007). Additionally, mothers with heterozygous genotype (CT) had an increased risk of SPTB (OR = 1.67, 95% CI [1.09 – 2.57], p < 0.02). However, because of few individuals (n = 2) that were detected to carry the TT genotype in the current study, its association with SPTB was in conclusive as the model failed to resolved.

The TT genotype frequency, though very low (0.004) among all the women (**Table 4.9**), was also found only in women with SPTB, specifically, those with PPROM (**Table 4.11**). It was not thus not surprise that a further test of association between the *SERPINH1* (- 656 C/T) SNP and the two types of SPTB (PPROM and SPTL) found the T allele to significantly increased the risk of PPROM (OR = 1.9, 95% CI [1.09 – 2.46], p < 0.01). Among the other SNPs examined, mothers with heterozygous genotype (1G2G) of the *MMP-1* (-1607 1G/2G) had a suggestive risk of SPTB (OR = 1.34, 95% CI [0.97 – 1.86], p < 0.08).

Table 4.8: Univariate allelic association with SPTB

Locus	Allele A/B[α]	SPTB women versus Term women		*p*-value
		OR	95% CI	
MMP-1 (– 1607)	1G/2G	1.13	0.89 – 1.44	0.32
EEFSEC rs2955117	G/A	0.95	0.74 - 1.22	0.70
AGTR2 rs5950491	C/A	1.19	0.83 - 1.69	0.35
ADCY5 rs9861425	A/C	0.95	0.72 – 1.25	0.71
SERPINH1 (– 656)	C/T	1.63	1.08 – 2.46	0.018

[α] = B is the risk allele

Table 4.9: The genotype frequencies associated with SPTB and term births.

Locus	Genotype	SPTB n (frequency)	Term Birth n (frequency)	Total n (frequency)	p-value
MMP-1 (-1607 1G/2G)	1G1G	63 (0.26)	90 (0.32)	153 (0.29)	0.25
	1G2G	124 (0.51)	123 (0.44)	247 (0.47)	
	2G2G	58 (0.24)	67 (0.24)	125 (0.24)	
EEFSEC rs2955117 (G/A)	GG	85 (0.35)	98 (0.35)	183 (0.35)	0.59
	AG	128 (0.52)	138 (0.49)	266 (0.51)	
	AA	32 (0.13)	44 (0.16)	76 (0.15)	
AGTR2 rs5950491 (C/A)	CC	182 (0.74)	217 (0.78)	399 (0.76)	0.60
	AC	55 (0.22)	56 (0.20)	111 (0.21)	
	AA	8 (0.03)	7 (0.03)	15 (0.03)	
ADCY5 rs9861425 (A/C)	AA	11 (0.05)	18 (0.06)	29 (0.06)	0.33
	AC	108 (0.44)	107 (0.38)	215 (0.41)	
	CC	126 (0.51)	155 (0.55)	281 (0.54)	
SERPINH1 (- 656 C/T)	CC	186 (0.76)	236 (0.84)	422 (0.80)	0.039*
	CT	57 (0.23)	44 (0.16)	101 (0.19)	
	TT	2 (0.008)	0 (0.000)	2 (0.004)	

*Chi-Squared test of trend

Table 4.10: Genetic model description of risk of association of genotypes with SPTB

Locus	Model	Comparison	SPTB women versus Term women		
			OR	95% CI	*p*-value
MMP-1-1607	Dominant	2G2G + 1G2G versus 1G1G	1.29	0.89 - 1.87	0.18
	Recessive	2G2G versus 1G2G + 1G1G	0.90	0.64 - 1.26	0.54
	Additive	1G1G versus 1G2G versus 2G2G	1.21	0.93 - 1.43	0.19
	Heterozygous	1G2G versus 1G1G + 2G2G	1.34	0.97 - 1.86	0.08
EEFSEC rs2955117 (G/A)	Dominant	AA + AG versus GG	0.97	0.68 - 1.37	0.85
	Recessive	AA versus AG + GG	0.78	0.54 - 1.13	0.19
	Additive	GG versus AG versus AA	1.00	0.79 - 1.27	0.97
	Heterozygous	AG versus GG + AA	1.18	0.85 – 1.62	0.32
AGTR2 rs5950491 (C/A)	Dominant:	AA + AC versus CC	1.03	0.73 - 1.44	0.86
	Recessive	AA versus AC + CC	0.81	0.54 - 1.33	0.48
	Additive	CC versus AC versus AA	1.20	0.81 - 1.20	0.28
	Heterozygous	AC versus AA + CC	0.83	0.55 – 1.26	0.39
ADCY5 rs9861425 (A/C)	Dominant	CC + AC versus AA	1.08	0.78 - 1.49	0.64
	Recessive	CC versus AA + AC	0.74	0.48 - 1.13	0.17
	Additive	AA versus AC versus CC	1.04	0.84 - 1.29	0.69
	Heterozygous	AC versus AA + CC	0.89	0.64 – 1.24	0.49
SERPINH1 - 656 C/T	Dominant	TT + CT versus CC	1.27	0.89 - 1.80	0.18
	Recessive	TT versus CT + CC	2.51×10^{6}	6.93×10^{-37}-NA	0.98
	Additive	CC versus CT versus TT	1.77	1.17 – 2.71	0.007
	Heterozygous	CT versus CC + TT	1.67	1.09 – 2.57	0.02

*=Only two SPTB women had TT genotypes. No control/Term women had TT genotype

Table 4.11: Univariate *SERPINH1* (– 656) allelic association between birth outcomes

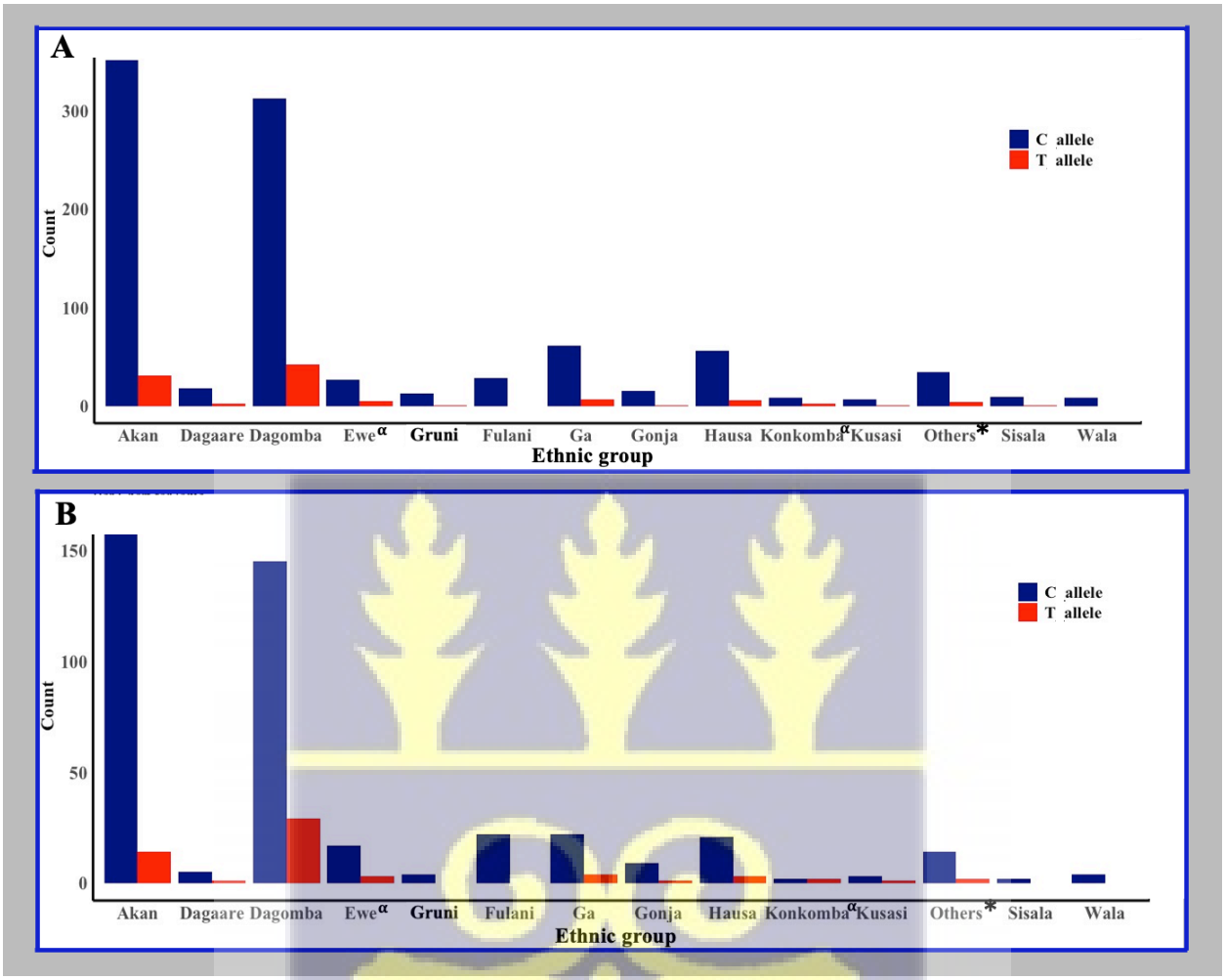
Locus	Genotype	Type of birth (Counts)		Allele A/B[α]	Risk metrics		
PPROM versus SPTL							
		PPROM	SPTL		OR	95% CI	p-value
SERPINH1 (– 656)	CC	70	116	C/T	1.29	0.75 – 2.21	0.36
	CT	23	34				
	TT	2	0				
PPROM versus Term							
		PPROM	Term				
SERPINH1 (– 656)	CC	70	236	C/T	1.9	1.09 – 2.46	0.01
	CT	23	44				
	TT	2	0				
SPTL versus Term							
		SPTL	Term				
SERPINH1 (– 656)	CC	116	236	C/T	1.50	0.94 – 2.40	0.10
	CT	34	44				
	TT	0	0				

[α] = B is the risk allele

4.7.5. Ethnic variation of *SERPINH1*-656 C/T in Ghanaian women

The distribution of *SERPINH1*- 656 C/T SNP varied among the various ethnic groups involved in the study as shown in **Figure 4.18, panel A (Table AIX of Appendix A).** The T allele, the risk allele frequency, ranged from 0.20 among the Konkomba group to 0.00 among the Fulani and the Wala ethnic groups. The T allele frequencies among the Ewe and Konkomba ethnic groups were however inflated by TT homozygous genotype found only among these two ethnic groups. Considering the women with SPTB alone as demonstrated in **Figure 4.18, panel B (Table AX of Appendix A)**, the influence of the TT homozygous genotype was evidenced in the allele frequencies of the Ewe and Konkomba ethnic groups as it increased their T allele frequencies.

SPTB among the Fulani, Gruni, Sisala and Wala ethnic groups were however not influenced by the T allele because all their participants with SPTB had CC homozygous genotypes.



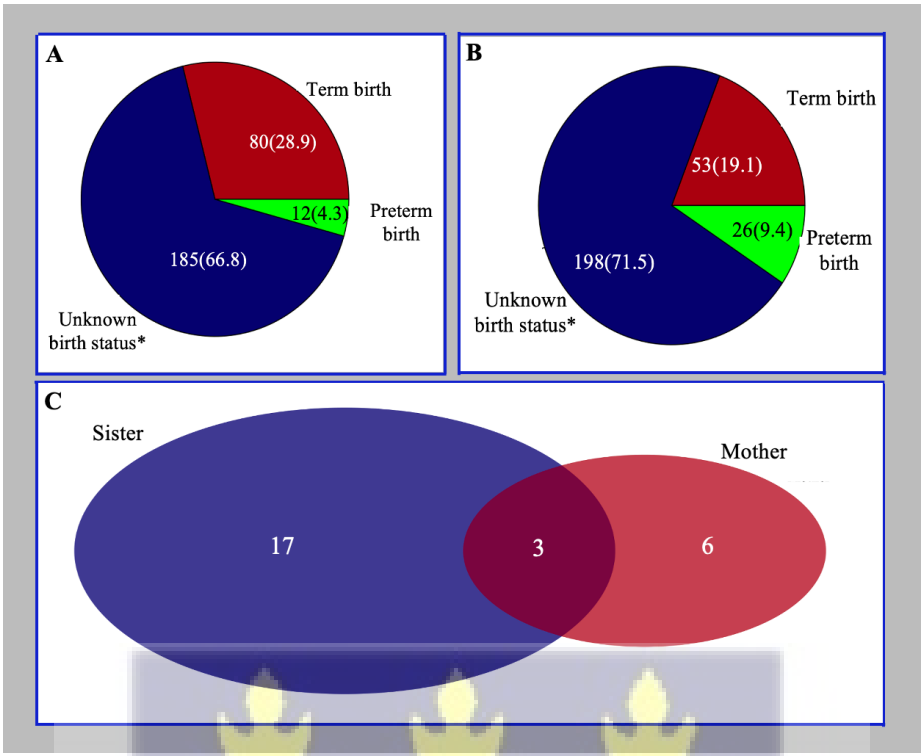
Key

Other* = other ethnic groups (Banda, Barsari, Bimoba, Builsa, Dangbe, Grusi, Guruma, Kotokoli, Krobo, Lobi, Mamprusi, Mosi, Mole, Sefwi, Wangara and Zabrama which had a maximum of three participants each at the recruitment stage. $^{\alpha}$ represent ethnic groups with *SERPINH1*- 656 TT genotype.

Figure 4.18: Ethnic distribution of SERPINH1- 656 C and T alleles in Ghanaian women*. Panel **A** illustrates the distribution of C and T alleles among the ethnic groups, panel **B** shows the ethnic distribution of the alleles among the women with SPTB.*

4.8. Awareness of family history of preterm birth.

The result of the SPTB mothers' awareness of whether they were born term or preterm as well as their family history of preterm birth is summarised in **Figure 4.20**. Almost 67% (185/277) of the postpartum women with SPTB did not know whether they were born preterm or term. Approximately 4% (12/277) of those with knowledge of their personal birth history responded that they were born preterm (**panel A**). The response of postpartum mothers with SPTB about their knowledge of whether their mothers and/or sisters (if any) was born preterm or term is shown in **panel B**. Among these participants, over 70% (198/277) did not know whether their mother and/or sister(s) was born preterm. However, about 9% responded that either their mother or sister or both were born preterm. The response of the SPTB mothers based on the number of their mothers and/or sisters who were born preterm illustrated on a Venn diagram (**panel C**). Three each of the respondents had their mothers and sisters were born preterm. Seventeen of the respondents had their sisters alone born preterm and 6 had their mothers alone born preterm.



Unknown birth status* = Mothers do not know whether they were born preterm or term

Figure 4.19: Awareness of family history of preterm birth among SPTB participants. *Respondent with knowledge of whether they were born term or preterm* ***(panel A)****. The respondents' knowledge of whether their mothers and/or sisters (if any) was born preterm or term is shown in* ***panel B****. The response of the SPTB mothers based on the number of their mothers and/or sisters who were born preterm illustrated on a Venn diagram* ***(panel C)****.*

4.9. Expression of selected genes in SPTB and term placental tissues.

The fold change gene expression levels of *COL4A1*, *COL4A2*, *COL4A3*, *P4H* and *SERPINH1* are demonstrated in **Figure 4.21**, panels **A** – **E**, respectively from women with TT, CT and CC genotypes of *SERPINH1* (- 656 C/T) in a cases-control assessment. The TT genotypes were from placental tissues of only cases. In an ascending order of fold change of *SERPINH1* expression levels, TT was the least of 0.77, followed by CT cases with 0.94. The CT controls, CC cases and CC control had fold changes of 1.07, 1.20 and 1.05 respectively. However, a one-way ANOVA (p = 0.12) did not show any statistical difference ($p < 0.05$) in the gene expression levels among the genotypes. Likewise, there was no statistical difference between the *SERPINH1* (- 656 C/T) genotypes and the expression of *COL4A1*, *COL4A2*, *COL4A3* and *P4H*.

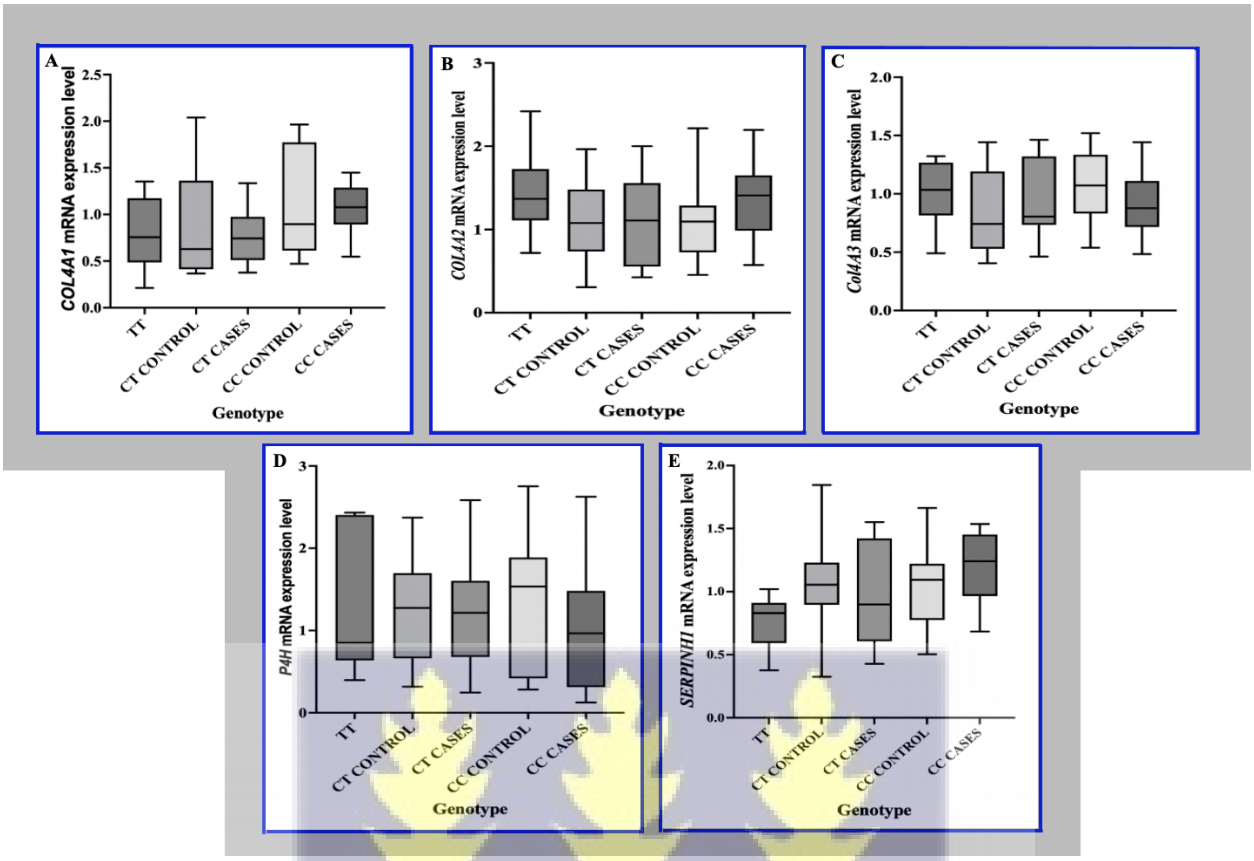


Figure 4.20: Expression of SERPINH1 (-656 C/T), COL4 and P4H in SPTB in term placental tissues. *Panels **A** – **E** demonstrates the fold change gene expression levels of COL4A1, COL4A2, COL4A3, P4H and SERPINH1. The TT, CT and CC are the SERPINH1 (- 656 C/T) genotypes of the tissues used for the assessment of the expression of each of the genes.*

CHAPTER FIVE

5.0. DISCUSSION

This study sought to investigate maternal characteristics including exposures, attitudes and genetic markers associated with SPTB among Ghanaian women as well as neonatal characteristics resulting from SPTB. The study is of an extreme public health importance due to the high prevalence of preterm birth in the country and the huge socioeconomic and health impact associated with preterm birth.

The study sites were strategically distributed across Ghana in order to capture women with diverse ethnic, sociocultural, socioeconomic and geographical backgrounds. The report of the 2010 Ghana Population and Housing Census provides 67 ethnic groups across 216 districts (Ghana Statistical Service, 2013). Generally, women participants from 29 ethnic groups (**Figure 4.1**, **panel A**) took part in the study. Together, the Akan and the Dagomba ethnic groups constituted almost 70% of the study participants, which is not surprising because over 6 out of 10 Ghanaians belong to either the Akan or Mole Dagbani ethnic group.

5.1. Maternal age at delivery and SPTB.

The association between maternal age and rates of preterm birth in general is considered to be U-shaped where the risk increases at the lower ages, especially less than 20 years and ages higher than 35 years (Hidalgo-Lopezosa *et al.,* 2019). The main reason being the poor anatomical developmental at the adolescent to early adult ages to cater for the growing foetus as well as the predisposition to non-communicable health challenges like hypertension and diabetes from ages above 35 years predispose these age groups to preterm birth (Oxlund *et al.*, 2010). This study used age group (26-30 years), the age group at less SPTB risk as reference in the assessment of

the association between maternal age and SPTB as previously done in other similar studies (Bakker *et al.,* 2011; Marvin-Dowle *et al.,* 2018; Tembo *et al.,* 2020).

In this study maternal age less than 20 years was found to be independently associated with the risk of SPTB by 2-fold when compared against a reference age of 26-30 years (**Table 4.3**). This corroborates with a previous study which reported that pregnant women with age <20 years are almost twice likely to experience preterm birth as compared to the age group of 20 - 35 years. In addition, studies from different populations had similar observations. Possibly, these women had not reached their optimal state of anatomic development to cater for the developing foetus. According to Oxlund and colleagues, collagen concentration and its stiffness in the cervix increases with age. Collagen is the major constituent of the cervix (Oxlund *et al.,* 2010). Thus, possibly, the adolescent uterine collagen content is not adequate to sustain the pressure posed by the expanding volume of the developing foetus. Additionally, the competition for nutrition for growth for both the pregnant adolescent and the foetus could have compromised the nutrition for the foetus and resulted in SPTB similar to the adolescent sheep experiment conducted by Wallace et al. (Wallace, 2019; Wallace et al., 1996, 1999, 2010). Additionally, pregnant adolescent girls also have limited knowledge of accessing health care systems and do not receive antenatal services required to maintain a healthy pregnancy. Such women do not also get the required social support because most of them may be unmarried, unemployed and stigmatised. These factors may increase the stress levels, a factor associated with preterm birth in women (Blumenshine *et al.,* 2010). Lifestyle behaviours like smoking has been found to be independently associated with SPTB (Delcroix *et al.,* 2023). However, women with lifestyles involving alcoholism, and smoking were excluded from the current study. The finding however contradicts with a Chilean study that found age ≤ 19 years to be 30% protective against all

preterm birth subtypes compared to women 20 – 35 years. (Araya *et al.,* 2017). It is not surprising because Araya *et al.* included all birth such as multiple births as part of their multivariate model that included significant second- and third-order interactions. Multiple birth result in preterm birth much more that singletons, usually due to over distension of the uterus that result in early active uterine contraction and cervical insufficiency (Blencowe *et al.,* 2013; Seetho *et al.,* 2023), and its predominance increases with maternal age (Araya *et al.,* 2017; Otta *et al.*, 2016; Zapata-Masias *et al.,* 2016). Adolescents' less likelihood of delivering multiple babies as well as indicated preterm deliveries compared with adult women might have contributed to the outcome Araya and colleagues observed. The current study however excluded indicated preterm birth and multiple deliveries. It is thus not surprising that this current study did not find women above 35 years compared with women 26 – 30 years were not found to be associated with SPTB risk. The observation linking teenage pregnancy to SPTB suggests that government has to put policies in place to educate girls on the dangers of teenage pregnancies including SPTB.

5.2. Parity and SPTB

The study also assessed the number of instance(s) in which a woman has delivered a live neonate (at any gestation) or a foetus at 24 weeks and beyond (Maraj & Kumari, 2021) and the risk of SPTB. This study found that nulliparity was associated with SPTB risk by 64%. The association was however not significant after adjusting for antenatal visits and maternal age (**Table 4.3**). The risk of association is however supported by a population-based study which found a 95% risk (OR 1.95, 95% CI 1.89–2.00) associated with nulliparity and SPTB (Koullali *et al.,* 2020). It is similar to another population-based study conducted by Ananth and colleagues which reported a

10% increase in SPTB among women with no previous deliveries (Ananth *et al.,* 2007), and other studies (Berkowitz *et al.,* 1998; Lin *et al.,* 2021). The inability of the risk of association in the current study to be independent may be due to low samples size compared with the studies which found independent association. Ananth and colleagues for example studies with a sample size of 154, 810 – much higher the 604 used in the current study. The increased risk among nulliparous women may be due to the lack of pregnancy history which hinders the provision of a tailor-made service like cervical length screening, progesterone administration, cervical cerclage or pessary (Koullali *et al.,* 2020). Another reason could be that these nulliparous women did not seek the right medical attention at the appropriate time during pregnancy. Stigmatization especially reduce the willingness of adolescent women to seek the appropriate medical attention (Govender et al., 2020; Wiemann *et al.,* 2005). Furthermore, the link between nulliparity and SPTB was previously explained by reduced uteroplacental blood flow and a smaller uterine cavity in nulliparous women (Canteiro *et al.,* 2010; Wildemeersch *et al.,* 2013).

Other studies have found association between increasing parity, especially grand multiparity and SPTB. This is attributable to reduced cervical strength and ageing of the uterine environment due to maternal biological ageing. This predisposes the mothers to pregnancy related challenges like abruptio placentae, placenta previa and uterine rupture which may lead to SPTB (Agrawal *et al.,* 2011; Emechebe *et al.,* 2016). Notwithstanding, the role of parity may be context specific with effects of poverty combined with maternal stress, and other factors associated with preterm birth (e.g., age, education, and ethnicity) interacting to increase risk of preterm birth (Shaikh *et al.,* 2011).

5.3. ANC visits and SPTB

Antenatal care (ANC) provides health promotion, screening, and support to the expectant mother. Mothers attending regular ANC service get services including: nutritional interventions which encompasses dietary counselling and supplements; maternal and foetal assessment of anaemia, asymptomatic bacteriuria, intimate partner violence, gestational diabetes, HIV, syphilis, TB, tobacco use, dairy foetal movement counting, symphysis-fundal height measurement, antenatal cardiotocography, ultrasound scan and doppler ultrasound of foetal blood vessels. Other services include preventive measures such as: antibiotics for asymptomatic bacteriuria (ASB), antibiotic prophylaxis to prevent recurrent urinary tract infections, antenatal anti-D immunoglobulin administration, preventive anthelminthic treatment, tetanus toxoid vaccination, intermittent preventive treatment in pregnancy (IPTp) for malaria prevention and pre-exposure prophylaxis (PrEP) for HIV prevention. Additional services provided include: interventions for common physiological symptoms such as: nausea and vomiting, heartburn, leg cramps, low back and pelvic pain constipation, varicose veins and oedema (WHO, 2016).

The positive effects of ANC on perinatal outcomes, includes reduced rates of preterm labour, low birthweight and perinatal death (Gurung *et al.,* 2020; Pusdekar *et al.,* 2020). Finding from this study showed that ANC < 4 times during pregnancy was independently associated with 79% risk in SPTB after adjusting for maternal age and parity (**Table 4.3**). A similar result was observed in a cross-sectional secondary data analysis by Hidayat *et al.* (2016), who reported that patients who received antenatal care < 4 times were approximately two times (OR=1.865) greater chance to experience preterm birth compared to patients with ANC ≥ 4 times. Similarly, Abu Hamad and colleagues observed that the risk of preterm birth increased two folds among those who attended < 4 visits as compared to those who attended 4 and above visits during the

entire pregnancy period (Abu Hamad *et al.,* 2007). Similar results were obtained elsewhere (Fagbamigbe *et al.,* 2021; Sarker *et al.,* 2020). A factor that could confound antenatal attendance is timely visit. According to Sarker and colleagues, the number of visits plus timely visits enhances the overall benefits of antenatal care by about 2 folds (Sarker *et al.,* 2020). These measures explain why women who visited ANC 4 times and above are at low risk of SPTB. The critical role ANC cannot be overemphasised, and more public educations is needed to encourage pregnancy women to target the recommended visits to avoid adverse pregnancy related outcomes including preterm birth. The observation made in this study suggests that young, unmarried mothers have to be supported through social protection schemes or targeted antenatal care. A targeted antenatal care that focuses antenatal services on women's individual needs and risk profiles, rather than giving the same schedule and interventions to all pregnant women could reduce the risk of adverse pregnancy outcomes

5.4. Marital status and SPTB

Marital complexities are not homogenous. Different marriages influenced by family structure and the type of marriage have different turn of events (Raj, 2019; Tenkorang, 2023). However, being married at the time of pregnancy and child delivery have been shown to be protective against all types of preterm birth (Farbu *et al.,* 2014; Luo *et al.,* 2004; Merklinger-Gruchala & Kapiszewska, 2019). In this study it was found that unmarried status is associated with increased SPTB risk by over 70%. However, the risk was not independent when it was adjusted for maternal age (**Table 4.3**).

The unadjusted risk corroborates several larger population studies (El-Sayed *et al.,* 2012; Kamburova *et al.,* 2016; P. Zhang *et al.,* 2023). Plausible explanation to this comes mainly from

maternal health, especially, mental health. Psychosocial stresses have been identified as risk factors associated with SPTB (Yaya *et al.,* 2021). Usually, pregnancies from unmarried women are more likely to be unintended and in turn can have negative consequences on both the woman and the growing foetus (Mohd Zain *et al.,* 2015). Increased social support from home, especially from the husband tends to buffer the stresses pregnant women go through. They get the protective corollaries of marriages like social support and financial support (Gondwe *et al.,* 2017). It is thus not surprising, based on Polish study that found preterm birth risk increases from 10% among married women with husbands present to 90% among unmarried women without husbands (Merklinger-Gruchala & Kapiszewska, 2019).

Young unmarried women, especially adolescents tend to bear the brunt of such a scenario. They become stressed because of uncertainty of their relationship with the unborn child's father and their ability to cater for the child. Additionally, they usually are unemployed, not independent and have reduced antenatal care which culminates in SPTB (Brink *et al.,* 2020). This suggests that marriage provides a safety net against adverse pregnancy such. Even among well-established common law unions where the partners live together and have legal rights like legally married couples, preterm birth risk is 14% higher among mothers in such unions compared with traditional marriage relationships (Luo *et al.,* 2004). This suggests that marriage provides a safety net against adverse pregnancy outcomes including preterm birth, suggesting that that young, unmarried mothers have to be supported through social protection schemes to reduce the stress on these women which ends up with adverse pregnancy outcomes such as SPTB.

However, Further research is warranted to explore whether the relationship between marital and SPTB varies across age groups, or whether age acts purely as a confounder. Stratified analyses or interaction terms could be useful in uncovering more nuanced relationships.

5.5. HB at booking and SPTB

The study further assessed the another modifiable risk, serum haemoglobin (Hb) concentration which is associated with several adverse pregnancy outcomes. (Klebanoff *et al.*, 1991). Considering the number of women with moderate to mild anaemia, (**Table 4.3**) 53% of Ghanaian pregnant women had anaemic (Hb < 11g/dl at sea level) at their first hospital visit. This was is less than the 56% previously reported in the West Gonja District of Ghana (Tibambuya et al., 2019). However, this prevalence prevalence is much higher than the 50.8% in the Northern part of Ghana (Wemakor, 2019) and the 40.8% reported in Sunyani, Ghana (Anlaakuu & Anto, 2017). The difference is likely due to their study design. The two studies were both cross sectional and the pregnancy period that the study was conducted was not stated. Conversely, this current study focused on the serum iron levels at booking to capture the levels anaemia status at the initiation of pregnancy. It was also meant to reduce the tendency of including serum iron levels that have been influenced by the use of prophylaxis or iron deficiency therapy by the pregnant woman.

It is also noteworthy that the mean Hb level at booking of mothers with SPTB found to be 10.67 ± 1.00 g/dl, statistically lower (p = 0.004) than their counterparts in the term group (10.9 ± 0.96) (**Table 4.3**). This corroborates several studies (Burden *et al.,* 2023; Figueira *et al.,* 2021; Kemppinen *et al.,* 2021; Ohuma *et al.,* 2023). What was intriguing was that mild anaemia (Hb 10 – 10.9 g/dl), a range below the recommended 11.0 g/dl was not associated with the risk of SPTB. However, moderate anaemia [Hb (9.9-7.0) g/ dl at sea level)] was significantly associated with increased the risk of SPTB by 60% (p = 0.03). This association was however not independent [OR 1.46 (95% C1 0.94 - 2.26, p = 0.09)] (**Table 4.3**). after adjusting for antenatal visits. This suggests that deteriorating maternal haemoglobin levels beginning from moderate anaemia may

be associated with SPTB among pregnant women which corroborates a study in Saudi Arabia which recorded increase prevalence of preterm birth from 9% among women with moderate anaemia to 15% among women with severe anaemia (< 7 g/dl) (Aboushamat & Nanu, 2016). It also suggests that at mild Hb levels (Hb 10 – 10.9 g/dl), Ghanaian women are able to provide the necessary nourishment needed to sustain pregnancy till term.

Notwithstanding, there are myriads of explanation that link anaemia to SPTB. This includes the fact that anaemia impair oxygen supply to the foetus due to reduced oxygen-carrying capacity of the blood resulting in foetal hypoxia and foetal stress. This result in increased synthesis of corticotrophin-releasing hormone (CRH) potentially triggering preterm birth via the foetal HPA axis (Irwin *et al.,* 2021). Additionally, maternal anaemic status reduces their immunity predisposes them to infections. Maternal infections especially, ascending infection have been linked to SPTB (Kemppinen *et al.,* 2021; Nsereko *et al.,* 2022). However, infection is possibly not a factor in this study because women with record of infection or identified to have infection during interviewing at recruitment were excluded. Maternal anaemia could also interfere with nutrient transport to the growing foetus due to impaired uteroplacental blood flow. This deprives the foetus of the required nutrition triggering physiologic stress in the foetus (Silver *et al.,* 2011; Wang *et al.,* 2022) This can cause the foetus to release stress hormones that could trigger SPTB (Chumak & Grjibovski, 2010). Other attributable factors include poor nutrient intake (Seid *et al.,* 2023) as well as haemoglobinopathies such as sickle cell anaemia (Oteng-Ntim *et al.,* 2015) and thalassemia (Traisrisilp *et al.,* 2017).

This study however contradicts with studies conducted in Tanzania (Stephen *et al.,* 2018) and southern India (Rao *et al.,* 2018) which did not find any association between maternal haemoglobin levels and preterm birth. The difference might have resulted from the fact that these

two studies included all forms of preterm birth cases. Thus, both SPTB and indicated preterm births which might have brought about the difference. However, it strengthens the fact that maternal African women, possibly may have a peculiar physiological way of sustaining pregnancy till term even in a mild anaemia status.

5.6. Age at menarche and SPTB

This study also investigated the association between age at menarche and SPTB. It was observed that generally, age at menarche was not associated with SPTB. However, age at menarche (<13 years) was identified to be associated with 42% increased risk of SPTB even though not statistically significant (**Table 4.3**). This suggests that earlier menarche (≤13) may have a biological risk associated with SPTB. However, further investigation is required. What makes this aspect of the study unique is that studies focusing on the association between age at menarche and SPTB is extremely scanty. The other studies have focused on preterm in general. Since the categories of preterm, thus, spontaneous preterm birth and indicated preterm birth are triggered by different pathologies, the influence of age at menarche on each of the preterm birth phenotypes may be different.

Nonetheless, a couple of studies have found age at menarche to be associated with preterm. Earlier, Li and colleagues found age at menarche ≤11 years to be associated with 67% increased the risk of preterm birth (Li *et al.,* 2017). This current study did not use very low age at menarche because of sample size of the participants within this age range. The effect of age at menarche on preterm birth is divergent. While early onset has been associated with preterm birth, late onset has also been found to be protective. This is evidenced in a Japanese cohort study which found that age at menarche (≥15 years) to reduce the risk of preterm birth by 21%. The

complexity of preterm birth aetiology is evidenced in age at menarche-preterm birth research. An example is study by Kanno and colleagues which did not find any association between early age at menarche (≤9 years) and preterm birth (Kanno *et al.,* 2022).

Lower age at menarche is influences increased production of sex hormones, particularly oestradiol which could surge maternal systemic inflammation and increase SPTB risk. This is evident in the increased amniotic fluid oestradiol concentration among women with preterm birth (Mazor *et al.,* 1994). Increased serum oestradiol triggers the expression of oxytocin receptor, prostaglandin receptors and stimulates prostaglandin synthesis via the synthesis of COX-2. These processes further quicken myometrial contractility and labour (Behrman & Butler, 2007).

5.7. Maternal psychological distress and SPTB

Outcome from this study based on the Centre for Epidemiological Studies-Depression (CES-D) scale found that possible depression ($16 \geq$ CES-D score ≤ 19) was associated with SPTB among Ghanaian women. It was found to independently increase the risk of SPTB by at least 86% after adjusting for and occupation (**Table 4.4**). The link between maternal psychological distress and SPTB has been attributed to the amplification of maternal hypothalamic-pituitary-adrenal (HPA) axis activities leading to increased serum cortisol which could trigger SPTB. Large population based longitudinal studies have also associated maternal psychological distress and preterm birth (Class *et al.,* 2011; Sigalla *et al.,* 2017).

The detection of this psychological state and SPTB may be due to increased risk of maternal age less than 20 years to SPTB identified in this study. Most of these women go through extreme psychological stresses due to relationship uncertainties (Nyadanu *et al.,* 2022). Additionally, many of these young women ae not gainfully employed and taking care of the un-born baby as

well as themselves places psychological burden on then that could predispose them to SPTB. Wemakor & Mensah, (2016) found maternal psychological distress to be associated with almost 2.5-fold increased risk of childhood stunting, suggesting that maternal stress predisposes the foetus to nutritional insults that is also associated with preterm birth (Lis *et al.,* 2023). Maternal psychological stress also reduces placental weight due to under nourishment and compromised immunologic and metabolic process. The reduced placental weight compromises foetal nourishment. This could lead to foetal oxidative stress and potentially SPTB (Tegethoff *et al.,* 2010)

5.8. Placental compromise and SPTB

The placenta controls vital metabolic, respiratory and endocrine processes during pregnancy. It also safeguards the foetus from infections and environmental influences throughout gestation. Compromised placental task could activate a range of pregnancy complications, including SPTB (Matoba *et al.,* 2021). SPTB attributed to placental dysfunction can result from factors such as intrauterine infection or inflammation, and foetal and maternal malperfusion (Sprong *et al.,* 2023) which compromises placental function, increasing oxidative stress in the foetus and triggering SPTB (Ericksen *et al.,* 2020).

The present study found chorioamnionitis to be present in both SPTB and term placental tissues (**Figure 4.2**). Women with hospital record of infection were excluded from the study, as a result, identifying chorioamnionitis in the placental tissues of participants suggests that some of the women had asymptomatic infection that were not diagnosed. However, even though chorioamnionitis is mostly caused by ascending infection, it is worth noting that it may occur as a result of trauma which had disrupted the integrity of placental tissue resulting in the release of

damage-associated molecular patterns (DAMPs) or oxidative-stress-induced-damage that might have activated immune cells to release proinflammatory cytokines. This results in the placental invasion of neutrophils and macrophages (Menon *et al.,* 2020; Romero *et al.,* 2011). Studies on postpartum placental bacteriologic cultures also suggest that vaginal microflora contamination could give a false impression of chorioamnionitis resulting from bacterial contamination. (Berezowsky *et al.,* 2022). This study found the frequency of chorioamnionitis in placental tissues to increase with decreasing gestational age from 10% in tissues 34 – 36 gestational weeks to 25% in tissues < 28 weeks (**Figure 4.2, panel B**). which corroborate a previous study (Erdemir *et al.,* 2013). This suggests that chorioamnionitis could be an attributable risk factor to SPTB among women experiencing extreme SPTB.

Maternal malperfusions identified were: accelerated villous maturation, perivillous fibrin deposition, fibrinoid islands, distal villous hypoplasia and villitis of unknown alethiology (**Table 4.5** and **Figure 4.4**). Collectively, maternal malperfusion was detected in over 6 out 10 placental samples. This was higher than the 4 out of 10 detected by Jaiman and colleagues in the USA (Jaiman *et al.,* 2021). This may contribute to reasons why African women are predisposed to SPTB more than people of other races. It could have also resulted from different maternal environmental exposures like maternal stress that is associated with reduced placental weight and function (Tegethoff *et al.,* 2010). On the contrary, prevalence of the various measures of maternal malperfusion were similar to other studies (Brink *et al.,* 2022; Iskender *et al.,* 2017; Kulkarni *et al.,* 2021) Maternal malperfusion interferes with the feto-maternal circulation of nutrients and other physiological substances necessary to maintain pregnancy (Loukeris *et al.,* 2010). Reduced foetal nourishment activates the release of stress hormones that could trigger SPTB (Chehaibi *et al.,* 2016; W. Liu *et al.,* 2011; Plewes *et al.,* 2017).

5.9. Neonatal characteristics

Neonatal characteristics measured in the study include: birthweight, HC, length and Apgar score at both the first and the fifth minute. As expected, the term born children had significantly higher birth weight, HC, length and Apgar score at both the first and the fifth minute than the SPTB counterparts (**Figure 4.6**). This was partly because of the truncation of their gestational duration due to preterm delivery. With Ghana's preterm birth prevalence going as high as almost 19 % in tertiary health facilities (Adu-Bonsaffoh *et al.,* 2019), it suggests that many children in Ghana are deprived of the optimum gestational duration for proper growth and development. This study was a case-control study; hence we could not estimate the prevalence of SPTB in Ghana.

It is worthy of emphasis that the differences between the developmental milestones of the SPTB neonates and their full-term counterparts in the current study might have worsened because of the inclusion of livebirths below 28 weeks in our study. Almost 8% of the SPTB neonates included in the study were in the extreme preterm birth category (WHO, 2023). The fragile state of these neonates requires sophisticated intensive care units with the appropriate human resources in health facilities to properly transition them from the intrauterine to the extrauterine environment. This is what LMIC lack, leading the loss of many of search neonates (Blencowe *et al.,* 2013). Unfortunately, due to inadequate advanced techniques in handling extreme preterm neonates, the Ghana health service classifies all deliveries below 28 weeks as spontaneous abortion or miscarriage. It suggests that health care attention given to women in labour less than 28 gestation weeks in Ghana focuses primarily on saving the life of the mother rather than that of the neonate. It is plausible to suggest that that there are many families in Ghana that lose their babies from preterm related deaths due to this classification.

The anthropometries mentioned earlier (birthweight, HC, length) were also used to assess the intra uterine growth and development of Ghanaian children. It was realised that males generally had higher birthweight, head circumference, and length (**Figures 4.8 - 4.10**). This corroborates other studies (Olsen *et al.,* 2010; WHO, 2023). The medians of the anthropometries recorded in this study (**Tables AV to AX**) of **Appendix A** compared with that of foetal growth chart reported by Olsen and colleagues (Olsen *et al.,* 2010) suggests a divergence in the growth parameters between the Ghanaian and the Olsen foetal growth from the 29th weeks of gestation and continues with a consistent drop in the Ghanaian foetal anthropometries till delivery at term. Olsen and colleagues' study was useful for comparison because of its large sample size (257 855 neonates) with 15.7% of them being blacks or of African ancestry. Admittedly, about 50% and 24% of neonates whose data was used for the Olsen curve were of whites and Hispanics origin. As a result, differences observed in the current study and the Olsen study could have been influenced by genetics (Alyaqoub *et al.,* 2012). Additionally, several other factors including socioeconomic and maternal attitudes during pregnancy may have resulted in the difference(Christian, 2022).

A major bottleneck to optimal foetal growth and development is poor antenatal care. It was observed in this study that poor antenatal visits < 4 predisposed mothers to SPTB. Similarly several studies in Ghana have reported poor antenatal visits to increase the risk of adverse pregnancy outcome including preterm birth and low birth weight deliveries even at term (Adam *et al.,* 2019; Adu-Bonsaffoh *et al.,* 2019; Agbozo *et al.,* 2016; Banchani & Tenkorang, 2020). Mothers attending regular antenatal visits enable them to get the needed interventions like iron supplements and prophylaxis needed to maintain optimal foetal growth and development (Afaya *et al.,* 2021).

Reduced foetal weight along the gestational period is an indication of an acute reduced foetal nourishment, especially, poor nutrient intake. However, chronic poor foetal nourishment affects foetal HC as well as their length (Belkacemi *et al.,* 2010; Gokhale & Rao, 2021; Kabahenda & Stoecker, 2024). This may also result from a compromised placental function due to poor development (Uwimana *et al.,* 2023; Wright *et al.,* 2023), as well as reduced maternal nutritional intake. Nutrition for optimal foetal growth is essential even at the pre-pregnancy stage. During pregnancy, additional nutrients in addition to the pre-pregnancy intakes is required to for foetal growth and development (Marshall *et al.,* 2022; Wrottesley *et al.,* 2015). Unfortunately, nutritional insecurity affects vulnerable people like pregnant women in Sub-Saharan Africa (Ankrah Twumasi et al., 2023; Cassimon et al., 2023).

Infections especially, malaria (Uwimana *et al.,* 2023; Wright *et al.,* 2023), pre-existing maternal health conditions like diabetes, hypertension and thyroid disorders (Adu-Bonsaffoh *et al.,* 2017; Beneventi *et al.,* 2022; Dassah *et al.,* 2019) and maternal lifestyle behaviour like smoking and alcohol abuse (Broccia et al., 2023; Delcroix et al., 2023) could result in intrauterine growth restriction and impair foetal development. It is worth noting that mothers with such conditions were excluded from the study.

Another important measure of neonatal health at birth that the current study considered were the 1st and 5th minute Apgar scores **(Figure. 4.7**). The percentage of neonates with very low Apgar score in the 1st minute (Apgar score 0 – 3) was 7% , higher than the 6.5% recorded by Dassah and colleagues at a tertiary hospital in Ghana (Dassah *et al.,* 2014). This could be attributed to the inclusion of neonates with lower gestational ages (24 to 27) weeks into the current study. Reduced Apgar score has been associated with increased risks of mortality and neurodevelopment, 2-fold increased risk of cardiovascular diseases in early adulthood and 76%

increased risk of attention deficit hyperactive disorder (ADHD) in children (Bala *et al.,* 2023; Razaz *et al.,* 2023; Shah *et al.,* 2022).

Unfortunately, the information on the socio-economic burden of preterm birth on families and the national resources in Ghana is very scanty. It would have given the citizens a better outlook on the devastating impact of preterm birth on child development in the country. However, the increased prevalence suggests that massive investments in human resource training and infrastructure is needed to take care of children born preterm.

5.10. Genetics of SPTB and gestational duration in the Ghanaian woman

Genetics has long been found to be associated with SPTB based on intergenerational studies (Bhattacharya *et al.,* 2010; Kistka *et al.,* 2007; Mercer *et al.,* 1999; Plunkett *et al.,* 2009; Smid, Jong, *et al.,* 2017; Treloar *et al.,* 1999), candidate gene investigations (Sheikh *et al.,* 2016; Song *et al.,* 2017), genome-wide association studies (GWAS) (Huusko *et al.,* 2018). A pivotal aspect of the current study sought to determine the association between genetic markers and SPTB and genetic association among Ghanaian women. Four genetic loci (*AGTR2* rs5950491, *WNT4* rs56318008, *EEFSEC* rs2955117 and *ADCY5* rs9861425) identified in the European genome-wide association studies to be associated with gestational duration (Zhang *et al.,* 2017) as well as *SERPINH1*- 656 (C/T) and *MMP1* (-1607 1G/2G) found to increase the risk of PPROM, a type of SPTB among African Americans via candidate gene studies.

This study did not replicate the association between four loci (*AGTR2* rs5950491, *WNT4* rs56318008, *EEFSEC* rs2955117 and *ADCY5* rs9861425) and gestational duration identified in the European genome-wide association studies (Zhang *et al.,* 2017). This could partly be attributed to the inter-population differences in the risk allele that modify gestational duration

and susceptibility SPTB. An observation that was intriguing is that in the current study the *WNT4* rs56318008 was found to be monomorphic with the CC genotype among all the 525 women genotyped. This observation, though puzzling, is not surprising because population-based studies have found the *WNT4* rs56318008 T allele frequency to be 1% in African populations but 14% in European population (Ensembl, 2024). In fact, the *WNT4* rs56318008 (C/T) has been found to be monomorphic with the CC genotype among some African sub-populations like the: Esan in Nigeria; African ancestry in South West United States; Gambians in the West Division of The Gambia; Luhya in Webuye, Kenya; Mendes in Sierra Leone; and the Yoruba in Ibadan, Nigeria (Ensembl, 2024). This clearly suggests that the minor T allele of *WNT4* rs56318008 that alters gestational duration among the European population could be population specific and possibly absent in many indigenous African communities.

This suggests that the contribution of the *WNT4* rs56318008 T allele to the over 2-day increase in gestational duration observed in the European population (Zhang *et al.,* 2017) could not be benefited by Ghanaian women. This could contribute the reasons associated with the increased preterm birth rates among people of African descent even after adjusting for socio-economic, environmental and socio-cultural factors (Martin *et al.,* 2017; Smid, *et al.,* 2017). This a huge benefit to the European population because the lesser the gestational age, especially below 37 weeks, the more vulnerable the neonate to the extra-uterine environment (Blencowe *et al.,* 2013).

Although the association between the three loci three loci *AGTR2* rs5950491, *EEFSEC* rs2955117 and *ADCY5* rs9861425 gestational duration in the current study was not statistically significant ($p > 0.05$), an interesting observation was a consistent pattern of effect of the loci on gestational duration similar to the findings of Zhang and colleagues. In the current study, the loci *ADCY5* rs9861425 and *AGTR2* rs5950491 had negative effect sizes (-0.59, $p = 0.78$; and -3.03, p

= 0.22) respectively; while *EEFSEC* rs2955117 had a positive effect size (0.25, p = 0.89). Zhang and colleagues also found the loci *ADCY5* rs9861425 and *AGTR2* rs5950491 with negative effect sizes at both discovery and replication stages (*ADCY5* rs9861425 had effect size -0.60, p = 6.1 x 10^{-7} at discovery stage and -1.38, p = 9.5 x 10^{-6} at the replication stage) and (*AGTR2* rs5950491 had effect size -0.83, p = 6.8 x 10^{-11} at discovery stage and -1.75, p = 4.7 x 10^{-8} at the replication stage). Notably, Zhang and colleagues also found the loci *EEFSEC* rs2955117 with a positive effect size at both discovery and replication stages (0.99, p = 1.5 x 10^{-11}) at discovery stage and -1.91, p = 7.6 x 10^{-7} at the replication stage) (Zhang *et al.,* 2017). This suggests a possibility of the loci having a similar effect on gestational duration. The outcome of the current study may have not reached statistical significance based on sample size. Zhang and colleagues genotyped over 40,000 women while the current study genotyped 525 women. It may also be due to variation in the risk allele frequencies across different populations. The risk allele frequencies observed in the current study for *EEFSEC* rs2955117, *ADCY5* rs9861425 and *AGTR2* rs5950491 were 0.4, 0.74 and 0.87 respectively (**Table 4.7**), while Zhang and colleagues had the risk allele frequencies of 0.289, 0.453 and 0.423 for *EEFSEC* rs2955117, *ADCY5* rs9861425 and *AGTR2* rs5950491 respectively (Zhang *et al.,* 2017). The differences in the frequencies are also evident on the Ensemble.org database (Ensembl, 2024). Additionally, the lack of association of gestational duration loci in Ghanaian women, despite GWAS hits in Europeans, highlights the need for more inclusive, ancestry-specific research.

The role of a collagenase like MMP1 in the degradation of fibrillar collagen resulting in the turnover of the extracellular matrix during parturition prompted its consideration in this study. The integrity of collagen is crucial in the maintenance of the foetal membrane as well the uterus to sustain pregnancy till term. The contribution of *MMP1,* influenced by the *MMP1* (-1607

1G/2G) SNP to pathophysiological conditions associated with disproportionate degradation of collagen such as aneurysm (abnormal localised dilation of blood due to weakening of the blood vessels) (Gorący *et al.*, 2020), stroke (Chehaibi *et al.,* 2014), as well as congenital anomalies (Djuric *et al.*, 2014) and PPROM (Fujimoto *et al.,* 2002) demonstrates the impact of the SNP on diverse health outcomes.

Even though the minor 2G allele of *MMP1* (-1607 1G/2G) has been attributed to several pathological states, the current study however, did not find it to be associated with SPTB among Ghanaian women. On the other hand, the 1G2G heterozygous genotype provided a suggestive risk to SPTB but not reach statistical significance. A plausible reason for the inconsistencies in expression by the *MMP1* (-1607 1G/2G) SNP could be due to an epigenetic marker identified to be located at (-1538) of the *MMP1* promoter that also influences *MMP1* expression. According to Wang and colleagues, a hypomethylation of the epigenetic marker also increases *MMP1* expression different from the *MMP1* (-1607 1G/2G) SNP in cultured amnion fibroblast (Fujimoto et al., 2002; Wang *et al.,* 2008). The amnion is a principal component of the foetal membrane; thus, it becomes unquestionable to suggest that the epigenetic marker plays a crucial role in the increased compartmentalized expression of *MMP1* in the amnion, resulting in PPROM. It may also be due to the sample size of the current study.

A striking observation made in the current study is that the *SERPINH1*- 656 (C/T) SNP which has already been identified to be associated with PPROM among African-Americans (Wang *et al.,* 2006) was also found to be a marker associated with SPTB in Ghanaian women. In the current study, each T allele – the risk allele in the *SERPINH1*- 656 (C/T) SNP was found to decrease gestational age by 2.35 days, $p < 0.02$ (**Table 4.7**). Additionally, the T allele was found to increase the risk of SPTB by 63%. (**Tables 4.8**). Under the additive model, each additional T

allele increased the risk of SPTB by 1.7 times, suggesting the crucial role the T allele could play in SPTB predisposition. It is not surprising that the women who had the TT genotype had SPTB (**Table 4.10**). This gives much impetus to the reason why the women identified in this study to be monomorphic for the *SERPINH1*- 656 (C/T) SNP, with the TT genotype had SPTB. However, care must be given to this interpretation because there were only 2 women with TT genotype in the current study. A larger study is needed to confirm the frequency of the TT genotype in the population.

Further, a comparative analysis of the T allele of *SERPINH1*- 656 (C/T) SNP, focusing on the two SPTB birth outcomes (PPROM and SPTL) and term birth (control) found the T allele of *SERPINH1*- 656 (C/T) SNP to increase risk of only PPROM by 90% ($p < 0.01$). Rather, the T allele was not found to increase the risk of SPTL (**Table 4.11**). This suggests that the T allele of *SERPINH1*- 656 (C/T) SNP increases the risk of PPROM rather than SPTB in general. What was also unambiguous was that both women with the TT genotype had PPROM (**Table 4.11**), further suggesting that the T allele of the *SERPINH1*- 656 (C/T) SNP is much more associated with PPROM. This confirms an earlier study by Wang and colleagues that the T allele of *SERPINH1*- 656 (C/T) SNP increases the risk of PPROM in African Americans (Wang *et al.,* 2006). Additionally, it is worth noting that the 63% increase in risk of the T allele of *SERPINH1*- 656 (C/T) SNP to the occurrence of SPTB (**Table 4.8**) in the current study is lower than about the 3-fold risk at a significance level of up to $p < 0000045$ recorded in earlier studies of an association between the T allele of *SERPINH1*- 656 (C/T) SNP and PPROM, a type of SPTB (Wang *et al.,* 2006). The difference in the experimental design in both studies is that the current study used on maternal samples (maternal venous blood) whiles Wang and colleagues used on foetal samples (umbilical cords, cord blood, or neonate cheek swabs). It suggests that the SNP has a much more

foetal effect compared with maternal effect. This could confirm Wang and colleagues' observation that the T allele *SERPINH1*- 656 (C/T) SNP had a reduced promoter activity in amnion fibroblast, different from its activity in dermal fibroblasts and uterine smooth muscle cells.

It is known that the T allele of the *SERPINH1*- 656 (C/T) reduces gene promoter activity of the *Heat shock protein 47* gene and reduce collagen synthesis, an important extracellular component in tissues like the foetal membrane needed to sustain pregnancy to term. Pregnancy demands a significantly stronger foetal membrane to accommodate foetal growth. Studies show a remarkable eight-fold increase in collagen content in the pregnant human uterus at term compared to the non-pregnant state (Morrione & Seifter, 1962). This suggests that any disruption in the collagen synthesis process could be deleterious to the sustenance of pregnancy.

Another noticeable observation was that the T allele frequency varied along ethnic lines in Ghana. The T allele frequency observed in this study was 0.1, a little lower than the range of (0.11 to 0.24) observed in some African American ethnic groups of West African Ancestry (Wang *et al.,* 2006) as well as the 0.33 in African Americans (ethnicity not specified) (Rocnik *et al.,* 2002). Even though people of African ancestry is have been found to have higher T allele frequency compared with the Caucasians (Rocnik *et al.,* 2002; Wang *et al.,* 2006), an observation from this study suggests that some ethnic groups in Africa may have lower T allele frequency similar to other races with lower T allele frequency. An example is the Fulani ethnic group where all the 14 women from this ethnic group genotyped were monomorphic for *SERPINH1*- 656 (C/T) SNP, with the CC genotype. A larger study is needed to further assess the frequency of the monomorphic genotype in the ethnic group. Interestingly, 11 of these 14 women had SPTB. This additionally suggests that women's genetic predisposition to SPTB in Ghana and

possibly Africa may vary among ethnic groups, and varied maternal genetic and environmental exposures apart from the *SERPINH1*- 656 (C/T) SNP increases their risk to SPTB. This suggests that further genetic assessment, a comprehensive one like whole genome study in addition to a rigorous maternal exposure assessment may be needed to understand inter-ethnic differences in maternal and foetal predisposition to SPTB in Ghana and Africa whole.

In this study, another feature of the *SERPINH1*- 656 (C/T) SNP worth noting was the very low genotype frequency (0.004) of the TT genotype compared with the 0.19 and 0.8 of the CT and the CC genotypes respectively among the participant (both cases and controls) recruited (**Table 4.9**). Since the T allele of the *SERPINH1*- 656 (C/T) SNP has an additive effect associated with PPROM, it suggests that a lot of infants with TT were born preterm. Unfortunately, inadequate infrastructure in the form of neonatal intensive care units as well as the human resource to cater for these fragile neonates may have led to increased mortality among neonates with this genotype. Notwithstanding, previous studies suggest that a further study of the TT genotype and survival of neonates need further investigation. A study conducted by Rocnik and colleagues found that the T allele of the *SERPINH1*- 656 (C/T) SNP significantly reduce *SERPINH1* expression in the formation of collagen in vascular smooth cells (Rocnik *et al.,* 2002), suggesting that an additive effect of the TT genotype could exacerbate the reduced expression of the *SERPINH1* which could have effect on the offspring survival. This suggestion is against the backdrop of the death of foetal *SERPINH1*- null mice and the ruptured blood vessels of embryos due to reduction mature type I collagen production in these organisms (Nagai *et al.*, 2000).

Variability in expression of the *SERPINH1* based on the *SERPINH1*- 656 (C/T) SNP in different tissues have also been observed by different authors. The current study conducted an assessment of the expression of the TT genotype using the placental tissues from the women with SPTB

compared with the CT and CC genotypes from other women with SPTB and those term births. A lower TT genotype lower fold change compared with the CT the CC genotypes was observed. However, the change was not significant (**Figure 4.21**). It suggests that even though the *SERPINH1*- 656 (C/T) SNP has been found to influence *SERPINH1* expression, there is a possibility of a variation in expressivity of the *SERPINH1* in different individuals. The expression of *COL4A1*, *COL4A2*, *COL4A3* and *P4H* genes were added in the gene expression protocol because of their crucial roles in the collagen formation process upstream of the *SERPINH1* activity in the collagen synthesis process.

Earlier, Rocnik and colleagues found the T allele of *SERPINH1*- 656 (C/T) SNP to reduce the expression of *SERPINH1* in human vascular smooth muscle cells (Rocnik *et al.,* 2002). Later, however, Wang and colleagues found the T allele to reduce the expression of *SERPINH1* in amnion fibroblasts but not uterine muscle cells or dermal fibroblast which suggests the vulnerability of the foetal membrane to PPROM if the foetus carries T allele of *SERPINH1*- 656 (C/T) SNP. The variability of *SERPINH1* and maternal exposure could be worth studying. Example, further study incorporating maternal consumption of vitamin C, a co-factor for the enzymes: prolyl hydroxylase and lysyl hydroxylase that hydroxylates proline and lysine residues needed to stabilise the collagen fibres in the extracellular matrix could be an important addition in the study of the *SERPINH1* activity in individuals.

5.11. SPTB women awareness of their personal or immediate family birth history

Preterm birth occurrence has been observed in intergenerational studies (Kistka *et al.,* 2008; Treloar *et al.,* 1999; York *et al.,* 2013). This suggests that a person's knowledge on whether he or she was born preterm as well as whether any of the close family was born preterm is extremely

crucial information for early detection of preterm birth risk factors. Especially, SPTB which is life-threatening, particularly in areas with poor access to health services.

Previous works have mainly depended on well-documented repositories with information of maternal and family pregnancy outcomes and health records to get such information (Kistka *et al.,* 2008; Treloar *et al.,* 1999; York *et al.,* 2013). It has however been observed that pregnancies of daughters born to mothers who were themselves born SPTB had 50% increased odds of delivering preterm babies (Bhattacharya *et al.,* 2010).

In this study 2 out of 3 women with SPTB did not know whether they were born preterm or term. Over 7 out of 10 women with SPTB did not know whether either their mother and/or sister(s) was born preterm or term **(Fig. 4.19)**. A review of the relevant literature did not provide any previous studies address the impact of such awareness on SPTB risk modification in Ghana. However, the findings from this study suggests that many women are not getting the benefits of risk profiling based on such knowledge. Knowledge of such family and personal history is expected to raise flags and allow health professionals and mothers alike to take the needed steps to ameliorate the anticipated risk.

5.12. Conclusions and recommendations

Generally, it is evident from the study that SPTB among Ghanaian women is mediated by a complex interplay of factors, including genetic predisposition, maternal health behaviours, prenatal care, and psychosocial well-being. These factors could have influenced the neonatal outcomes as well.

Maternal age less than 20 years, along with poor antenatal visits (less than 4 visits), emerged as significant risk factors of SPTB, independently associated with SPTB by approximately two-

fold. Additionally, depression, in the category of "possibly depressed" also independently emerged as a significant predictor of SPTB risk among Ghanaian women by approximately 80%. Nulliparity, moderate haemoglobin levels at booking and unmarried status were associated with increased risk of SPTB, although the association were not independent.

Even though participants did not have hospital record of infection, chorioamnionitis was detected in more than one out of ten placental tissues. However, the occurrence was not statistically different among placental tissues from women with SPTB and those with term birth. Placental malperfusion was detected in 6 out of 10 SPTB placental tissues. However, distal villous hypoplasia was not detected in the placenta from women with extreme SPTB.

Potential differences in foetal growth patterns were detected from reduced neonatal anthropometric measurements that deviated from the Olsen median curve from 29 weeks' gestation. Almost one out of ten of the neonates had very low Apgar score, largely attributed to extreme prematurity, but improved to less than 5 % in the fifth minute.

The genetic predisposition component was highlighted by genetic variations, such as the T allele of *SERPINH1*-656 (C/T) which was associated with over 2 days decrease in gestational duration and a 63% increased risk of SPTB. The *SERPINH1*-656 (C/T) SNP was also associated SPTB in the heterozygous and additive genetic models. Notably, The TT genotype frequency in the Ghanaian women population was very low at less than half a percentage, and was found only among those with SPTB, specifically women with PPROM. Comparing the two SPTB outcomes, with term birth, the T allele was associated with increased risk in PPROM but not SPTL.

A reduction in placental gene expression was observed among women with the TT genotype, though no statistically differences were observed compared to the CT and CC genotypes in both SPTB and term women. Generally, the *MMP1*-1607(G/GG) SNP was not associated with

increased SPTB risk. However, based on the heterozygous genetic model, the SNP appeared to provide a suggestive risk to SPTB as it did not reach statistical significance.

Some polymorphisms associated with gestational duration may be racially biased because polymorphisms such as *WNT4* rs56318008 (C/T), *ADCY5* rs9861425 (A/C), *AGTR2* rs5950491 (C/A), *EEFSEC* rs2955117 (G/A) identified to be associated with gestational duration among European women in a GWAS were not associated with gestational duration among Ghanaian women. Considering *WNT4* rs56318008, none of the Ghanaian women involved in the study had the risk T allele. Only the CC genotype was observed.

It is noteworthy that at least two-thirds of Ghanaian women with SPTB are unaware of their own birth status, and over seventy percent are unaware of their family members', indicating a potential lack of understanding of familial predisposition to preterm birth.

A multi-omic study encompassing: genomics, transcriptomics, proteomics, metabolomics as well as the study of the microbiomes of women with SPTB will provide a very comprehensive understanding of the aetiology of SPTB in Ghana and Africa. However, this approach of genetic studies is more resource intensive and requires with genomics institutes or research consortia and international support. Additionally, the lack of association of gestational duration loci in Ghanaian women, despite GWAS hits in Europeans, highlights the need for more inclusive, ancestry-specific research.

Additionally, a comprehensive transgenerational study of embedded with lifestyle characteristics is needed to better understand how SPTB runs through families in Ghana and Africa as a whole.

A larger nationwide population-based study is needed to provide much information about the foetal growth dynamics of Ghanaians. A nationwide foetal growth monitoring integrated into

routine antenatal services and national health surveys could help provide better information about the growth parameters of Ghanaian foetuses for appropriate remedial action if necessary. Public health awareness of its increased prevalence SPTB among the teenage girls, stressing on the ramifications associated with delivering preterm babies could encourage the youth, especially the girls to focus on building their carrier. This will reduce the tendency of girls becoming pregnant in their formative stages that could result in SPTB.

5.13. Limitations of the study

This study presents some limitations that must be acknowledged. First, the statistical power of the of the *SERPINH1* TT genotype was limited due to its low frequency (less than 0.5%) in the study population to detect robust associations, potentially underestimating its true effect on spontaneous preterm birth (SPTB). Although some genetic associations were observed, the restricted number of SNPs analysed mostly drawn from candidate genes and prior studies in non-African populations limits the comprehensiveness of genetic risk characterization in this cohort. Broader genome-wide analysis would have been more suitable to identify novel or population-specific variants relevant to Ghanaian women.

Second, most participants were unaware of their own or their family's preterm birth history, impeding exploration of hereditary contributions to SPTB risk. Although histological chorioamnionitis was detected in placental tissues, the absence of documented infection histories or microbiological confirmation precluded clear linkage between infections and SPTB. Furthermore, the study relied on self-reported sociodemographic and behavioral data (e.g., antenatal visits, depressive symptoms), which may be subject to recall bias or social desirability bias, affecting data accuracy. Additionally, histopathological analysis revealed malperfusion and

structural placental changes in some cases. However, about half (150/277) and (170/327) of the SPTB and term placental tissues underwent such evaluation, which may limit the generalizability of these observations.

Lastly, this study did not assess environmental exposures or epigenetic modifications, such as DNA methylation, which may significantly modulate gene expression and pregnancy outcomes. The lack of longitudinal follow-up and reliance on cross-sectional placental and neonatal assessments also limit insights into causal mechanisms underlying SPTB.

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Appendix A

Table AI: A table of the participants ethnic distributions

Ethnic group	Number of participants		
	SPTB	Term	Total
Akan	103(37.18)	125(38.23)	228(37.75)
Dagomba	90(32.49)	102(31.19)	192(31.79)
Ga	17(6.14)	25(7.65)	42 (6.95)
Hausa	14(5.05)	20(6.12)	34(5.63)
Ewe	12(4.33)	9(2.75)	21(3.48)
Fulani	11(3.97)	4(1.22)	15 (2.48)
Dagaare	4 (1.44)	7(2.14)	11(1.82)
Gruni	3 (1.08)	7(2.14)	10(1.66)
Konkomba	2(0.72)	4(0.92)	6(0.99)
Gonja	5 (1.81)	4(1.22)	9(1.49)
Kusasi	2(0.72)	3(0.92)	5(0.83)
Sisala	1(0.36)	4(1.22)	5(0.83)
Wala	3(1.08)	2(0.61)	5(0.83)
Other*	**10(3.61)**	**12(3.67)**	**22(3.64)**
Banda	0(0.00)	1(0.31)	1(0.17)
Barsari	0(0.00)	1(0.31)	1(0.17)
Bimoba	0(0.00)	1(0.31)	1(0.17)
Builsa	1(0.36)	0(0.00)	1(0.17)
Dangbe	1(0.36)	1(0.31)	2(0.33)
Grusi	0(0.00)	1(0.31)	1(0.17)
Guruma	1(0.36)	0(0.00)	1(0.17)
Kotokoli	2(0.72)	0(0.00)	2(0.33)
Krobo	1(0.36)	0(0.00)	1(0.17)
Lobi	0(0.00)	1(0.31)	1(0.17)
Mamprusi	1(0.36)	2(0.61)	3(0.50)
Mole	0(0.00)	1(0.31)	1(0.17)
Mosi	1(0.36)	1(0.31)	2(0.33)
Sefwi	1(0.36)	1(0.31)	2(0.33)
Wangara	1(0.36)	0(0.00)	1(0.17)
Zabrama	0(0.00)	1(0.31)	1(0.17)

Table AII: A tabular description of the neonatal characteristics of the SPTB and term groups.

Neonate Variable	SPTB n = 277			Term n = 327			*p* - value
	Mean ± SD	Median (IQR)	Range	Mean ± SD	Median (IQR)	Range	
Gestational age (Completed weeks)	32.56 ± 3.10	33 (31 - 35)	24 - 36	39.06 ± 1.40	39 (38 - 40)	37 - 42	<0.0001*
Birth weight (kg)	1.86 ± 0.60	1.90 (1.43 - 2.30)	0.50 - 3.20	3.08 ± 0.40	3.09 (2.80 - 3.45)	2.21 - 4.31	<0.0001*
H.C. (cm)	29.82 ± 2.93	29.80 (28.0 - 32.1)	21.70 - 35.90	33.53 ± 1.35	33.60 (32.70 - 34.45)	29.50 - 37.40	<0.0001*
Length (cm)	42.51 ± 4.61	43.30 (39.50 - 45.60)	29.20 - 52.60	49.60 ± 2.50	49.50 (48.00 - 51.40)	41.80 - 55.60	<0.0001*
Apgar score at 1 minutes	5.37 ± 1.58	6 (4 - 7)	2 - 8	7.77 ± 0.58	8 (7 - 8)	6 - 9	<0.0001*
Apgar score at 5 minutes	6.42 ± 1.78	7 (5 - 8)	1 - 9	8.72 ± 0.48	9 (8 - 9)	7 - 9	<0.0001*

H.C. = head circumference

Table AIII: Distribution of birth weights among Ghanaian male neonates per gestational age

Gestational age (Weeks)	n	Mean ± SD	Median (IQR)	Range
24	2	0.66 ± 0.57	0.66 (0.64 – 0.68)	0.62 – 0.70
25	2	0.93 ± 0.12	0.93 (0.89 – 0.98)	0.85 – 1.02
26	3	0.85 ± 0.25	0.84 (0.72 – 0.97)	0.60 – 1.10
27	4	1.01 ± 0.08	1.03 (0.97 – 1.07)	0.90 – 1.09
28	6	1.13 ± 0.22	1.15 (0.10 – 1.28)	0.80 – 1.40
29	8	1.18 ± 0.31	1.17 (0.90 – 1.35)	0.81 – 1.61
30	9	1.46 ± 0.30	1.50 (1.32 – 1.58)	0.85 – 1.90
31	11	1.58 ± 0.31	1.55 (1.39 – 1.76)	1.05 – 2.17
32	10	1.66 ± 0.20	1.67 (1.55 – 1.80)	1.30 – 1.92
33	15	1.89 ± 0.31	1.99 (1.69 – 2.10)	1.27 – 2.40
34	24	2.15 ± 0.21	2.13 (2.00 – 2.30)	1.70 – 2.50
35	25	2.27 ± 0.21	2.29 (2.16 – 2.38)	1.75 – 2.68
36	24	2.56 ± 0.40	2.61 (2.39 – 2.87)	1.40 – 3.20
37	24	2.89 ± 0.34	2.84 (2.65 – 3.12)	2.46 – 3.56
38	35	3.04 ± 0.41	2.95 (2.71 – 3.25)	2.50 – 4.00
39	40	3.16 ± 0.40	3.11 (2.90 – 3.48)	2.40 – 3.81
40	36	3.25 ± 0.44	3.20 (2.90 – 3.52)	2.56 – 4.30
41	18	3.32 ± 0.31	3.31 (3.11 – 3.52)	2.69 – 3.93
42	11	3.29 ± 0.31	3.27 (3.08 – 3.51)	2.75 – 3.67

Table AIV: Distribution of birth weights among Ghanaian female neonates per gestational age

Gestational age (Weeks)	n	Mean ± SD	Median (IQR)	Range
24	2	0.70 ± 0.21	0.70 (0.63 – 0.78)	0.55 – 0.85
25	4	0.76 ± 0.20	0.79 (0.67 – 0.88)	0.50 – 0.95
26	2	0.90 ± 0.12	0.91 (0.86 – 0.95)	0.82 – 0.99
27	3	0.93 ± 0.22	0.90 (0.81 – 1.03)	0.72 – 1.15
28	6	1.05 ± 0.13	1.08 (0.99 – 1.12)	0.84 – 1.20
29	9	1.28 ± 0.33	1.26 (1.14 – 1.42)	0.73 – 1.78
30	8	1.43 ± 0.33	1.45 (1.38 – 1.50)	0.92 – 1.90
31	10	1.56 ± 0.21	1.55 (1.45 – 1.71)	1.20 – 1.81
32	11	1.59 ± 0.30	1.55 (1.45 – 1.74)	1.20 – 2.26
33	14	1.72 ± 0.34	1.77 (1.56 – 1.98)	1.08 – 2.20
34	19	2.01 ± 0.22	2.00 (1.85 – 2.16)	1.62 – 2.41
35	22	2.29 ± 0.33	2.22 (2.00 – 2.50)	1.70 – 2.50
36	24	2.50 ± 0.32	2.57 (2.33 – 2.68)	1.79 – 3.11
37	26	2.80 ± 0.37	2.72 (2.53 – 3.02)	2.21 – 3.50
38	36	2.95 ± 0.30	2.86 (2.74 – 3.22)	2.40 – 3.60
39	46	3.01 ± 0.41	3.00 (2.70 – 3.28)	2.30 – 3.94
40	32	3.17 ± 0.30	3.16 (3.00 – 3.30)	2.60 – 3.98
41	16	3.19 ± 0.36	3.20 (3.00 – 3.42)	2.50 – 3.80
42	7	3.24 ± 0.52	3.16 (2.91 – 3.30)	2.77 – 4.31

Table AV: Distribution of head circumference among Ghanaian male neonates per gestational age

Gestational age (Weeks)	n	Mean ± SD	Median (IQR)	Range
24	2	22.25 ± 0.78	22.25 (21.98 – 22.53)	21.70 – 22.80
25	2	24.20 ± 0.99	24.20 (23.85 – 24.55)	23.50 – 24.90
26	3	23.70 ± 0.56	23.80 (23.45 – 24.00)	23.10 – 24.20
27	4	25.00 ± 1.12	24.85 (24.48 – 25.38)	23.80 – 26.50
28	6	26.22 ± 1.22	26.30 (25.28 – 26.58)	24.90 – 28.20
29	8	26.86 ± 0.80	26.75 (26.40 – 27.13)	25.80 – 28.50
30	9	27.92 ± 0.75	27.90 (27.40 – 28.30)	26.70 – 28.30
31	11	29.13 ± 1.80	28.70 (28.30 – 30.40)	26.00 – 31.80
32	10	29.36 ± 1.10	29.10 (28.83 – 29.50)	28.00 – 31.80
33	15	30.71 ± 1.33	30.80 (30.05 – 31.65)	27.50 – 32.70
34	24	31.08 ± 1.54	30.95 (30.15 – 32.50)	27.60 – 33.60
35	25	32.05 ± 1.46	32.20 (31.50 – 32.90)	28.70 – 34.50
36	24	32.81 ± 1.25	32.60 (32.28 – 33.65)	30.60 – 36.20
37	24	32.93 ± 1.42	33.20 (31.98 – 33.73)	29.50 – 35.50
38	35	33.42 ± 1.37	33.50 (32.55 – 34.30)	30.50 – 36.20
39	40	33.89 ± 1.10	33.80 (33.08 – 34.70)	31.60 – 35.60
40	36	34.32 ± 1.39	34.20 (33.45 – 35.05)	31.70 – 37.10
41	18	34.42 ± 1.14	34.25 (33.73 – 34.83)	32.80 – 37.40
42	11	34.01 ± 1.24	34.10 (33.60 – 34.90)	31.10 – 35.30

Table AVI: Distribution of head circumference among Ghanaian female neonates per gestational age

Gestational age (Weeks)	n	Mean ± SD	Median (IQR)	Range
24	2	22.45 ± 0.07	22.45 (22.43 – 22.48)	22.40 – 22.50
25	4	23.18 ± 0.87	23.00 (22.55 – 23.63)	22.40 – 24.30
26	2	24.20 ± 0.85	24.20 (23.90 – 24.50)	23.60 – 24.80
27	3	24.60 ± 0.53	24.80 (24.40 – 24.90)	24.00 – 25.00
28	6	25.52 ± 1.38	25.45 (24.43 – 26.55)	23.90 – 27.30
29	9	26.77 ± 1.02	26.50 (26.10 – 27.40)	25.50 – 28.30
30	8	27.41 ± 0.82	27.30 (27.00 – 27.88)	26.40 – 28.80
31	10	28.03 ± 1.23	28.05 (27.48 – 28.35)	25.70 – 30.00
32	11	29.23 ± 1.15	29.20 (28.25 – 29.95)	27.60 – 29.80
33	14	29.74 ± 1.47	29.65 (29.20 – 30.45)	27.30 – 32.60
34	19	30.64 ± 1.72	30.30 (29.55 – 31.55)	27.70 – 33.50
35	22	32.10 ± 1.84	32.00 (30.65 – 33.60)	29.00 – 35.40
36	24	32.23 ± 1.30	32.15 (31.68 – 32.73)	30.20 – 35.80
37	26	32.81 ± 1.42	32.65 (31.80 – 33.50)	30.70 – 36.10
38	36	32.94 ± 1.25	32.95 (32.28 – 33.33)	30.10 – 35.30
39	46	33.33 ± 1.19	33.55 (23.30 – 34.20)	29.90 – 35.50
40	32	33.74 ± 1.19	33.75 (32.98 – 34.53)	31.00 – 36.10
41	16	33.84 ± 0.96	33.80 (33.08 – 34.48)	31.80 – 35.30
42	7	33.67 ± 0.93	33.80 (32.80 – 34.50)	32.60 – 34.70

Table AVII: Distribution of length of Ghanaian male neonates per gestational age

Gestational age (Weeks)	n	Mean ± SD	Median (IQR)	Range
24	2	31.10 ± 2.69	31.10 (30.15 – 32.05)	29.20 – 33.00
25	2	34.75 ± 2.76	34.75 (33.78 – 35.73)	32.80 – 36.70
26	3	34.53 ± 2.02	34.20 (33.45 – 35.45)	32.70 – 36.70
27	4	35.25 ± 2.02	35.65 (34.25 – 26.65)	32.61 – 37.10
28	6	37.52 ± 1.69	37.55 (36.63 – 38.70)	35.00 – 39.60
29	8	36.90 ± 1.83	36.90 (35.83 – 37.75)	34.10 – 39.80
30	9	39.93 ± 3.32	40.20 (36.90 – 41.90)	36.30 – 45.30
31	11	41.48 ± 2.74	41.00 (40.05 – 43.60)	35.80 – 45.10
32	10	41.30 ± 2.32	41.70 (39.33 – 43.08)	37.50 – 44.70
33	15	43.82 ± 1.94	43.80 (42.50 – 44.85)	40.80 – 47.50
34	24	44.46 ± 2.40	44.10 (42.08 – 46.55)	41.10 – 49.60
35	25	46.06 ± 2.98	46.20 (44.20 – 48.10)	39.80 – 50.30
36	24	47.00 ± 2.96	47.10 (45.05 – 48.90)	39.70 – 52.60
37	24	48.67 ± 2.69	48.80 (47.60 – 49.90)	42.80 – 53.20
38	35	49.83 ± 2.38	49.70 (48.35 – 51.40)	43.90 – 54.10
39	40	50.00 ± 2.84	49.95 (48.45 – 51.90)	41.80 – 54.30
40	36	50.51 ± 2.63	50.55 (49.10 – 52.43)	44.60 – 53.60
41	18	50.79 ± 1.63	50.85 (49.73 – 52.23)	47.40 – 53.40
42	11	50.62 ± 2.71	50.60 (48.30 – 52.05)	47.10 – 54.70

Table AVIII: Distribution of length of Ghanaian female neonates per gestational age

Gestational age (Weeks)	n	Mean ± SD	Median (IQR)	Range
24	2	31.40 ± 1.13	31.40 (31.00 – 31.80)	30.60 – 32.20
25	4	32.73 ± 2.10	33.20 (31.78 – 34.15)	29.90 – 34.60
26	2	34.70 ± 1.70	34.70 (34.10 – 35.30)	33.50 – 35.90
27	3	34.67 ± 2.40	34.60 (33.45 – 35.85)	32.30 – 37.10
28	6	36.23 ± 2.20	35.95 (35.38 – 37.50)	33.00 – 39.30
29	9	37.21 ± 1.99	37.80 (36.80 – 38.10)	32.80 – 39.80
30	8	39.35 ± 2.50	39.65 (37.80 – 41.53)	35.70 – 42.30
31	10	40.73 ± 2.57	40.75 (38.50 – 42.73)	37.00 – 44.00
32	11	41.14 ± 2.87	40.90 (38.60 – 43.45)	37.80 – 46.70
33	14	42.83 ± 1.96	42.95 (42.08 – 44.30)	37.80 – 45.50
34	19	44.33 ± 1.77	44.30 (43.20 – 45.70)	40.50 – 47.40
35	22	45.42 ± 2.40	45.70 (43.73 – 47.08)	40.80 – 50.70
36	24	46.27 ± 2.65	46.25 (44.50 – 48.40)	41.00 – 50.60
37	26	47.85 ± 2.15	47.60 (46.80 – 48.30)	43.90 – 54.50
38	36	49.16 ± 2.10	49.25 (48.40 – 50.23)	44.30 – 55.20
39	46	49.43 ± 2.70	49.45 (47.80 – 51.20)	44.30 – 55.00
40	32	49.73 ± 1.85	49.70 (48.48 – 51.08)	46.40 – 52.90
41	16	50.14 ± 2.19	50.10 (48.40 – 51.43)	46.70 – 53.90
42	7	49.94 ± 2.25	49.70 (48.25 – 51.00)	47.50 – 53.90

Table AIX: Ethnic distribution of C and T alleles frequencies of *SERPINH1* - 656 C/T in Ghanaian women

Ethnic group	Genotype (count)			C allele	(T allele)	Total
	CC	CT	TT	n (frequency)	n (frequency)	
Akan	158	33	0	349 (0.92)	33 (0.08)	382
Dagomba	137	40	0	314 (0.89)	40 (0.11)	354
Ga	27	7	0	61 (0.90)	7 (0.10)	68
Hausa	25	6	0	56 (0.90)	6 (0.10)	62
Ewe	12	3	1	27 (0.84)	5 (0.16)$^{\alpha}$	32
Fulani	14	0	0	28 (1.00)	0 (0.00)	28
Dagaare	8	2	0	18 (0.90)	2 (0.10)	20
Gonja	7	1	0	15 (0.94)	1 (0.06)	16
Gruni	6	1	0	13 (0.93)	1 (0.07)	14
Sisala	4	1	0	9 (0.90)	1 (0.10)	10
Kusasi	3	1	0	7 (0.88)	1 (0.12)	8
Wala	4	0	0	8 (1.00)	0 (0.00)	8
Konkomba	4	0	1	8 (0.80)	2 (0.20)$^{\alpha}$	10
Others*	16	3	0	35 (0.92)	3 (0.08)	38

Other* = other ethnic groups (Banda, Barsari, Bimoba, Builsa, Dangbe, Grusi, Guruma, Kotokoli, Krobo, Lobi, Mamprusi, Mosi, Mole, Sefwi, Wangara and Zabrama which had a maximum of three participants each.

$^{\alpha}$ - inflated by at least 1 TT genotype

Table AX: Ethnic distribution of C and T alleles of *SERPINH1* - 656 C/T among Ghanaian women with SPTB

Ethnic group	Genotype (count)			C allele	(T allele)	Total
	CC	CT	TT	n (frequency)	n (frequency)	
Akan	72	15	0	159 (0.91)	15 (0.09)	174
Dagomba	58	29	0	145 (0.83)	29 (0.17)	174
Ga	9	4	0	22 (0.85)	4 (0.15)	26
Hausa	9	3	0	21 (0.88)	3 (0.22)	24
Ewe	8	1	1	17 (0.85)	3 (0.15)$^{\alpha}$	20
Fulani	11	0	0	22 (1.00)	0 (0.00)	22
Dagaare	2	1	0	5 (0.83)	1 (0.17)	6
Gonja	4	1	0	9 (0.90)	1 (0.10)	10
Gruni	2	0	0	4 (1.00)	0 (0.00)	4
Sisala	1	0	0	2 (1.00)	0 (0.00)	2
Kusasi	1	1	0	3 (0.75)	1 (0.25)	4
Wala	2	0	0	4 (1.00)	0 (0.00)	4
Konkomba	1	0	1	2 (0.50)	2 (0.50)$^{\alpha}$	4
Others*	7	1	0	15 (0.94)	1 (0.06)	16

Other* = other ethnic groups (Banda, Barsari, Bimoba, Builsa, Dangbe, Grusi, Guruma, Kotokoli, Krobo, Lobi, Mamprusi, Mosi, Mole, Sefwi, Wangara and Zabrama which had a maximum of three participants each.

$^{\alpha}$ - inflated by at least 1 TT genotype

Appendix B (CED-S scoring questions)

Table BI: A table of the CED-S scoring questions

	During the past week:	***Rarely*** or none of the time (less than 1 day)	***Some*** or a *little* of the time (1-2 days)	***Occasionally*** or a moderate amount of time (3-4 days)	***Most*** or all of the time (5-7 days)
1	I was bothered by things that usually don't bother me.				
2	I did not feel like eating; my appetite was poor.				
3	I felt that I could not shake off the blues even with help from my family or friends.				
4	I felt I was just as good as other people.				
5	I had trouble keeping my mind on what I was doing.				
6	I felt depressed.				
7	I felt that everything I did was an effort.				
8	I felt hopeful about the future.				
9	I thought my life had been a failure.				
10	I felt fearful.				
11	My sleep was restless				
12	I was happy				
13	I talked less than usual.				
14	I felt lonely				
15	People were unfriendly				
16	I enjoyed life				
17	I had crying spells				
18	I felt sad				
19	I felt that people disliked me.				
20	I could not get going.				

Table BII: A table of the CES-D scoring criteria

Scoring:	Rarely (Less than 1 day)	Some (1-2 days)	Occasionally (3-4 days)	Most (5-7 days)
Questions 4, 8, 12, and 16	3	2	1	0
All other questions	0	1	2	3

Appendix C **Questionnaire**

Title of Project: Genetic studies of spontaneous preterm birth in Ghanaian women

Participant Code _____ Interviewer_____

Part 1: Type of birth

1.1. Type of delivery ☐ Term delivery ☐ Preterm delivery **Please, if Term delivery, skip to 2.1**	**1.2.** Type of Spontaneous preterm birth ☐ Spontaneous preterm birth with intact membrane ☐ Preterm Premature Rapture of Membrane (**Please, tick)**

Part 2. Socio-demographic characteristics

2.1. Date of birth (D-M-Y) ___/___/___ Unknown ☐ (**Please, tick)**	**2.2.** Age: _____ Unknown ☐ (**Please, tick)**	**2.3.** Height: _____ cm
2.4. Ethnicity: ☐ Ga ☐ Akan ☐ Ewe ☐ Dagomba ☐ Hausa ☐ Sefwi. ☐ Other → Please specify_____ ☐ Unknown. (**Please, tick)**		**2.5.** Religion: ☐ Christianity ☐ Islam ☐ Traditional ☐ Other → Please specify: _____ ☐ Unknown. (**Please, tick)**
2.6. Highest level of education completed: ☐ No formal education ☐ Primary ☐ JSS ☐ SSS ☐ Tertiary → Please specify: _____ ☐ Other → Please specify: _____ ☐ Unknown. (**Please, tick)**		**2.7.** Marital Status: ☐ Married ☐ Single ☐ Divorced/Separated ☐ Widowed ☐ Other → Please specify: _____ ☐ Unknown (**Please, tick)**
2.8. Occupation: ☐ Unemployed ☐ Student ☐ Farmer ☐ Trader ☐ Artisan. ☐ Salaried worker → Please specify: ___ _____ ☐ Other → Please specify: _____ ☐ Unknown. (**Please, tick)**		**2.9.** Occupation of partner: ☐ Unemployed ☐ Student ☐ Farmer ☐ Trader ☐ Artisan. ☐ Salaried worker → Please specify: ____ _____ ☐ Other → Please specify: _____ ☐ Unknown. (**Please, tick)**

Part 3: Obstetric history

3.1. Number of children:	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ >4. (**Please, tick)**

3.1. Recurrent spontaneous pregnancy loss ☐ No ☐ Yes (**Please, tick**)	**3.2.** Type of pregnancy loss ☐ Miscarriage ☐ Stillbirth ☐ Other. Please mention the type_____ (**Please, tick**)

Part 4: Fertility history

4.1. Age at menarche (years)_____ ☐ Unknown	**4.2.** Number of children _____
4.3. Birth interval between start of current pregnancy and termination of previous pregnancy (months) _____	

Part 5: Preterm birth history

5.1. Were you born preterm? ☐ Yes ☐ No ☐ Unknown	
5.2. Was you mother, sister, cousin born preterm? ☐ Yes ☐ No ☐ Unknown **Please, *only*** *to be indicated if present at* ***maternal*** *side* If yes, ***specify*** (**tick + circle**)***:***	**5.3.** If 5.2 is yes, ***specify*** (**tick + circle**)***:*** ☐ Mother: **1** Validated **2.** Self-reported **99.** Unknown ☐ Sister: **1.** Validated **2.** Self-reported **99.** Unknown ☐ Cousin: **1.** Validated **2.** Self-reported **99.** Unknown ☐ Family member unknown

Part 6: History of Current birth

6.1. Number of ANC visits ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ >4	**6.2.** Gestational age at start of documented maternity care: _____(weeks + days)
6.3. **Earliest (the first)** ultrasound performed: ☐ No ☐ Yes → Date: ____ / ___ / ___	**6.4.** Gestational age at **earliest (the first)** ultrasound: _____ weeks + days ☐ Not applicable
6.5. Last menstrual period ☐ No ☐ Yes → Date: ____ / ___ / ___	**6.6.** BP at booking ☐ No ☐ Yes → Systolic: _____ mmHg Diastolic: _____ mmHg
6.7. BP at ANC ☐ No ☐ Yes → Systolic: _____ mmHg Diastolic: _____ mmHg	**6.8.** Hb **measured at booking** ☐ No ☐ Yes → Highest recorded: ____ Lowest recorded: _____ ☐ g/dL ☐ Other, ***please specify***: _____

Part 7: Maternal Psychological Stress Assessment

Please if unemployed, skip to 9.5		
7.1. How many hours do you do the work in a day? ☐ less than 4 hours ☐ 4-8 hours ☐ more than 8 hours	**7.2.** Does your work involve night shift? ☐ Yes ☐ No	**7.3.** Do you think that your work exceedingly stresses you? ☐ Yes ☐ No
7.4. Did you receive support from work place (e.g. leave to go to hospital) during pregnancy? ☐ All the time ☐ Sometimes ☐ Never	**7.5.** Is the father of your baby alive? ☐ Yes ☐ No **If Yes, please go to 9.7.**	**7.6.** Which period in pregnancy did you lose your baby's father? ☐ Within the first three months ☐ Within the second three months ☐ Within the last three months
7.7. How do you rate the support your baby's father gave you during pregnancy? ☐ poor ☐ satisfactory ☐ good ☐ very good ☐ excellent	**7.8.** Did you have money to take care of your living expenses (food, bills, rent, hospital bills) during pregnancy? ☐ All the time ☐ Some times ☐ Never	**7.9.** Did you lose any close relative (mother, father, sibling) during the pregnancy period) ☐ Within the first three months ☐ Within the second three months ☐ Within the last three months

7.10. Below is a list of ways you might have felt or behaved. Please indicate how often you felt this way during pregnancy (**Please, tick the most appropriate answer)**

	Rarely or none of the time	Some or little of the time	Occasionally or moderate amount of time	Most or all of the time
1. I was bothered by things that usually don't bother me.	☐	☐	☐	☐
2. I did not feel like eating	☐	☐	☐	☐
3. I felt that I could not overcome sadness even with help from my family or friends	☐	☐	☐	☐
4. I felt I was good as other people.	☐	☐	☐	☐
5. I had trouble keeping my mind on what I was doing.	☐	☐	☐	☐
6. I felt depressed.	☐	☐	☐	☐
7. I felt that everything I did was difficult for me	☐	☐	☐	☐
8. I felt hopeful about the future	☐	☐	☐	☐
9. I thought my life had been a failure.	☐	☐	☐	☐
10. I felt fearful	☐	☐	☐	☐
11. I had little sleep.	☐	☐	☐	☐
12. I was happy	☐	☐	☐	☐
13. I talked less than usual.	☐	☐	☐	☐
14. I felt lonely	☐	☐	☐	☐
15. People were unfriendly	☐	☐	☐	☐

	Rarely or none of the time	Some or little of the time	Occasionally or moderate amount of time	Most or all of the time
16. enjoyed life.	☐	☐	☐	☐
17. I had crying spells	☐	☐	☐	☐
18. I felt sad	☐	☐	☐	☐
19. I felt that people dislike me	☐	☐	☐	☐
20. I could not get going	☐	☐	☐	☐

SCORING: zero for answers in the first column, 1 for answers in the second column, 2 for answers in the third column, 3 for answers in the fourth column.

Part 8: Assessment of Neonate

8.1. Date of birth: ____ / ___ / ___	**8.2.** Gestational age _____ (days)
8.3. Head circumference _____ cm	**8.4.** Length _____ cm
8.5. Birth Weight _____ (grams)	**8.6.** First minute APGAR score_____
8.7. Fifth minute Apgar Score_____	**8.8.** Gender_____

Appendix D (Ethical approvals)

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. GHS/RDD/ERC/Admin/App 21/146
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Digital Address: GA-050-3303
Mob: +233-50-3539896
Tel: +233-302-681109
Fax + 233-302-685424
Email: ethics.research@ghsmail.org
14th May, 2021

Peter Nuro-Ameyaw
Department of Biochemistry, Cell and Molecular Biology
West Africa Centre of Cell Biology of Infectious Pathogens
P. O. Box LG 54
Legon

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC 009/02/21**
Study Title	Genetics Studies of Spontaneous Preterm Birth in Ghanaian Women
Approval Date	14th May, 2021
Expiry Date	13th May, 2022
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of a yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED..... Bannerman .....
Dr. Cynthia Bannerman
(GHS ERC Chairperson)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

In case of reply the number
And the date of this
Letter should be quoted

My Ref. No. KBTH/MD/03/2020

Your Ref. No.....

KORLE BU TEACHING HOSPITAL
P. O. BOX KB 77,
KORLE BU, ACCRA.

Tel: +233 302 667759/673034-6
Fax: +233 302 667759
Email: Info@kbth.gov.gh
pr@kbth.gov.gh
Website: www.kbth.gov.gh

21st April, 2020

PETER NURO-AMEYAW
DEP. OF BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY
WEST AFRICA CENTRE FOR CELL BIOLOGY OF INFECTIOUS PATHOGEN
LEGON

GENETIC STUDIES OF SPONTANEOUS PRETERM BIRTH IN GHANAIAN WOMEN

KBTH-IRB /00003/2020

Investigator: PETER NURO-AMEYAW

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to the study entitled: "Genetic studies of spontaneous preterm birth in Ghanaian women"

Please note that the Board requires you to submit a final review report on completion of this study to the KBTH-IRB.

Kindly, note that, any modification/amendment to the approved study protocol without approval from KBTH-IRB renders this certificate invalid.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 30th March, 2021. You are to submit annual report for continuing review.

Sincere regards,

DR. DANIEL ANKRAH
VICE CHAIR (KBTH-IRB)
FOR: CHAIR (KBTH-IRB

Cc: The Chief Executive Officer, KBTH

The Director of Medical Affairs, KBTH

KOMFO ANOKYE
TEACHING HOSPITAL

P. O. Box 1934
KUMASI - GHANA
Tel: +233 - 3220 - 22301- 4
Fax: +233 - 3220 - 24654/24621
Website: www.kathhsp.org

Our Ref. No: *KATH-IRB/AP/022/20*

Your Ref. No:.....

Komfo Anokye Teaching Hospital Institutional Review Board

Mr. Peter Nuro-Ameyaw,
Department of Biochemistry, Cell and Molecular Biology
P.O. Box LG 54,
Legon.

2nd June, 2020

Dear Mr. Nuro-Ameyaw,

Ethics Approval

Protocol title:	Genetic Studies of Spontaneous Preterm Birth in Ghanaian Women.
Study site:	Komfo Anokye Teaching Hospital, Obstetrics and Gynaecology Directorate.
Sponsor:	West Africa Centre for Cell Biology of Infectious Pathogens, University of Ghana.

We are writing in response to the clarifications and revised documents following review by the Komfo Anokye Teaching Hospital Institutional Review Board (KATH IRB) in respect of the research study referenced above.

We are pleased to inform you that KATH IRB, per your correspondence of 22nd April 2020, has given approval for the following study documents:

- Protocol *version 3 last updated 21st April 2020*
- Participants' Information leaflet and Informed Consent form *version 3 last updated 21st April 2020*
- Case Report Form *version 3 last updated 21st April 2020*

Approval for the study is in effect until 1st June, 2021 and it is the responsibility of the Principal Investigator to maintain the study in good standing at the Komfo Anokye Teaching Hospital. The Board anticipates to be notified of the actual start date of your project.

Prior to the expiration of the study approval, you must submit to the KATH-IRB an "Application for Continuing Review" along with provision of "Annual Report" when the study is ongoing, or a "Termination Report" if the research has been completed.

You must hastily report to the KATH-IRB should a modification to the research be proposed, and without delay if an unanticipated development occurs before the next required review. Regulations do not permit you to modify conduct of the study in its present form prior to ethics

approval; except where urgent action is required to eliminate an apparent immediate hazard to a study subject or other person. It is of utmost importance data generated from this study must be used for the intended purposes only.

Thank you.

Sincerely,

Prof. Kwabena Antwi Danso, BSc, MB ChB, FWACS, FGCS, FACOG
Chairman, KATH-IRB

UNIVERSITY OF GHANA

ETHICS COMMITTEE FOR BASIC AND APPLIED SCIENCES (ECBA

P. O. BOX LG 1195, Legon-Accra

Ref. No: ECBAS 007/19-20

18th October,2019.

Mr. Peter Nuro-Ameyaw
Department of Biochemistry, Cell and Molecular Biology
University of Ghana
Legon, Accra

Dear Mr. Nuro-Ameyaw,

ECBAS 007/19-20: GENETIC STUDIES OF SPONTANEOUS PRETERM BIRTH IN GHANAIAN WOMEN

This is to inform you that the above referenced study has been presented to the Ethics Committee for Basic and Applied Sciences for a full board review and the following actions taken subject to the conditions and explanation provided below:

Expiry Date:	01/10/2020
On Agenda for:	Initial Submission
Date of Submission:	02/09/2019
ECBAS Action:	Approved
Reporting:	Annually

Please accept my congratulations.

Yours sincerely,

Professor Daniel Bruce Sarpong
ECBAS Chairperson

ETHICS COMMITTEE FOR BASIC AND APPLIED SCIENCES
APPROVED
18 OCT 2019
UNIVERSITY OF GHANA

ETHICS COMMITTEE FOR BASIC AND APPLIED SCIENCES
APPROVED
18 OCT
UNIVERSITY OF GHANA