ASSOCIATION BETWEEN *CYP17A1* AND *HSD3B1* GENE POLYMORPHISMS AND TESTOSTERONE LEVELS IN NIGERIAN PROSTATE CANCER PATIENTS

EKENWANEZE, CHRISTOGONUS CHICHEBE
(22PCP02381)
B.Sc Biochemistry, Abia State University, Uturu,
Abia State.

AUGUST, 2024

ASSOCIATION BETWEEN *CYP17A1* AND *HSD3B1* GENE POLYMORPHISMS AND TESTOSTERONE LEVELS IN NIGERIAN PROSTATE CANCER PATIENTS

BY

EKENWANEZE, CHRISTOGONUS CHICHEBE
22PCP02381
B.Sc Biochemistry, Abia State University, Uturu,
Abia State.

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE, (M.Sc) IN BIOCHEMISTRY IN THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, NIGERIA

AUGUST, 2024

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Master of Science (M.Sc) in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria.

Miss Adefunke F. Oyinloye
(Secretary School of Postgraduate Studies) **Signature and Date**

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DECLARATION

I, EKENWANEZE, CHRISTOGONUS CHICHEBE, (**22PCP02381),** declare that this research is an original work carried out by me under the supervision of Professor O.O. Ogunlana in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota Nigeria. I attest that the thesis has not been presented wholly or partially for the award of any degree elsewhere. All sources of materials and scholarly publications used in the thesis have been duly acknowledged.

EKENWANEZE, CHRISTOGONUS CHICHEBE

Signature and Date

CERTIFICATION

This is to certify that the research work titled **"ASSOCIATION BETWEEN *CYP17A1* AND *HSD3B1* GENE POLYMORPHISMS AND TESTOSTERONE LEVELS IN NIGERIAN PROSTATE CANCER PATIENTS"** is an original work carried out by **EKENWANEZE, CHRISTOGONUS CHICHEBE (22PCP02381)** meets the requirements and regulations governing the award of Master of Science degree (M.Sc) in Biochemistry from the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria. It is approved for its contribution to knowledge and literary presentation.

Prof. Olubanke O. Ogunlana
(Supervisor) **Signature and Date**

Prof. Solomon O. Rotimi
(Head of Department) **Signature and Date**

Prof. Oluwatosin B. Adu
(External Supervisor) **Signature and Date**

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DEDICATION

This dissertation is dedicated to the Almighty God for His love and guidance throughout the cause of this project.

ACKNOWLEDGEMENT

I thank the Covenant Applied Informatics and Communication Africa Centre of Excellence (CApIC-ACE) for their invaluable support in funding my research. I am genuinely grateful for their belief in my work. Additionally, I wish to extend my deepest gratitude to my parents, Mr and Mrs A.E. Nwaigwe, my siblings Gaemezu, Zobam and Chijioke and nephew and nieces for their unwavering prayers, encouragement and generous financial support, which have been instrumental in making this journey possible. Thank you, Rev. Fr. Bartholomew Chukwu, for your spiritual and moral support.

I also want to thank all my lecturers, especially my supervisor, Prof. O.O. Ogunlana, for her enormous support and guidance throughout my project work. I especially appreciate the HOD and Cancer Genomic group coordinator, Prof. S. Rotimi, and CApIC_ACE Center Director, Professor E. Iweala, for their guidance and support towards my project.

To all members of Accountability Hub and Kingdom Professionals using Barr. Ngunan and Obinna and Mr Chukwunoso and Kenechukwu as points of contact, respectively; I am grateful for all the spiritual support, mentorship and encouragement.

Dear Dr Gwen Okeke, thank you for your unwavering support until your demise, and may your soul continue to rest in peace. Amen

Finally, I appreciate all my colleagues and friends I worked with and assisted each other, enabling me to conduct insightful research.

TABLE OF CONTENTS

CONTENTS	PAGES
COVER PAGE	**i**
TITLE PAGE	ii
ACCEPTANCE	**iii**
DECLARATION	**iv**
CERTIFICATION	**v**
DEDICATION	**vi**
ACKNOWLEDGEMENT	**vii**
TABLE OF CONTENTS	**viii**
LIST OF FIGURES	**xi**
LIST OF TABLES	**xiii**
ABSTRACT	**xiv**
CHAPTER ONE	**1**
INTRODUCTION	1
1.1 Background of the Study	1
1.2 Statement of Research Problem	2
1.3 Research Questions	4
1.4 Aim and Objective	4
1.5 Research Justification	4
CHAPTER TWO	**7**
LITERATURE REVIEW	7
2.1 Prostate Cancer	7
2.2 Development of Prostate Cancer	8
`2.2.1 Androgen Receptors	8
2.2.2 Testosterone	9
2.3 Single Nucleotide Polymorphism in Prostate Cancer	10
2.3.1 *CYP17A1* Polymorphisms and Prostate Cancer Risk	11
2.3.2 *HSD3B1* Polymorphisms and Prostate Cancer Risk	13
2.4 Prostate Cancer Therapies	17
2.4.1 Immunotherapy	17
2.4.2 Hormonal Therapy (Androgen Deprivation Therapy)	21
2.4.3 Chemotherapy	23
2.4.4 Radiotherapy	24

2.4.5 Cryotherapy 24

CHAPTER THREE **25**
MATERIALS AND METHODS 25
3.1 Materials 25
3.1.1 Equipment and Hardware 25
3.2.2 Reagents 25
3.2 Methodology 25
3.2.1 Study Population 25
3.2.2 Study Site 25
3.2.3 Inclusion and Exclusion Criteria 25
3.2.4 Sampling Technique 26
3.2.5 Ethics Approval 26
3.2.6 Blood Collection 26
3.3 Genotyping 26
3.3.1 DNA Extraction and Quantification 26
3.3.2 *CYP17A1* Genotyping 26
3.3.3 *HSD3B1* Genotyping 27
3.4 ELISA Analysis: 27
3.4.1 Quantification of Testosterone 27
3.4.2 Quantification of Androgen Receptors 27
3.5 Data Analysis 28

CHAPTER FOUR **29**
RESULTS 29
4.1 Genotyping Result for *CYP17A1* 29
4.1.1 Distribution of *CYP17A1* Genotypes 29
4.1.2 Distribution of *CYP17A1* Genotype among Groups 30
4.2 Genotyping Result for *HSD3B1* 31
4.2.1 Distribution of *HSD3B1* Genotypes 31
4.2.2 Distribution of *HSD3B1* Genotype among Groups 32
4.3 Levels of Hormone and Protein 33
4.3.1 Levels of Testosterone 33
4.3.2 Levels of Androgen Receptor 34

4.3.3 Testosterone and AR Levels 35
4.4 Association of between *CYP17A1* and *HSD3B1* Genotypes, Testosterone and Androgen Receptor 36
4.4.1 *CYP17A1* and Testosterone 36
4.4.2 *CYP17A1* and Androgen Receptor 36
4.4.3 *HSD3B1* and Testosterone 37
4.4.3 *HSD3B1* and Androgen Receptor 37

CHAPTER FIVE **38**
DISCUSSION 38

CHAPTER SIX **42**
CONCLUSION AND RECOMMENDATION 42
6.1 Summary 42
6.2 Conclusion 42
6.3 Contribution to Knowledge 42
6.4 Recommendation 43

REFERENCES **44**

LIST OF FIGURES

FIGURES **LIST OF FIGURES** **PAGES**

Figure 2.1: Incident and mortality rate of various cancers in men and prostate cancer 7

Figure 2.2: Prostrate shown in the male reproductive system 8

Figure 2.3: Pathway of testosterone 10

Figure 2.4: Androgen biosynthesis pathway in PCa 11

Figure 2.5: Forms of Structures of *CYP17A1* steroidal ligands 12

Figure 2.6: Disorders related to elevated *CYP17A1* expression and enhance steroid hormone synthesis. 12

Figure 2.7: Pathway showing inhibition of *CYP17A1* by abiraterone 13

Figure 2.8: Mechanism of *HSD3B1* (rs1047303) 15

Figure 2.9: Mechanism of PCa management after enzalutamide and abiraterone treatment 16

Figure 2.10: Mechanism of second-generation androgen synthesis inhibitors 16

Figure 2.11: Schematic representation of Checkpoint activities 18

Figure 2.12: Homologous delivery of CAR-T therapy 19

Figure 2.13: Mechanism of sipuleucel-T on Immune System 20

Figure 4.1: Graph showing the distribution of *CYP17A1* genotypes in the population 29

Figure 4.2. Bar chart distribution showing the *CYP17A1* genotype among control and case groups of PCa in the population 30

Figure 4.3: Graph showing the distribution of *HSD3B1* Genotypes in the population 31

Figure 4.4: Bar chart showing the distribution of *HSD3B1* genotype among control and case groups 32

Fig 4.5: Box plot illustrating testosterone levels distribution among prostate cancer and control 33

Figure 4.6: Box plot illustrating AR levels distribution among prostate cancer and control 34

Figure 4.7: Scatter plot illustrating testosterone and AR levels correlation among prostate cancer and control 35

Figure 4.8: Box plot illustrating *CYP17A1* and testosterone distribution among PCa and control 36

Figure 4.9: Box plot illustrating *CYP17A1* and AR distribution among PCa and controls 36

Figure 4.10: Box plot illustrating *HSD3B1* and testosterone distribution among PCa and controls 37

Figure 4.9: Box plot illustrating *HSD3B1* and AR distribution among PCa and controls 37

LIST OF TABLES

TABLES	LIST OF TABLES	PAGES
Table 4.1.	Frequency of Distribution of *CYP17A1* Allele and Genotype	30
Table 4.2.	Frequency of Distribution of *CYP17A1* Allele and Genotype	32

ABSTRACT

Prostate cancer (PCa) represents a significant worldwide health challenge, being the second most diagnosed cancer and a top cause of cancer-related deaths among men. African men exhibit high PCa incidence and mortality rates. Genetic variation in androgen pathways is essential in PCa development and progression. The Cytochrome P450 17A1 (*CYP17A1*) gene encodes a critical metabolic enzyme involved in testosterone (TT) synthesis, as it converts cholesterol into androstenedione. Similarly, the 3β-hydroxysteroid dehydrogenase type 1 (*HSD3B1*) gene encodes an enzyme that catalyses the conversion of dehydroepiandrosterone (DHEA) to androstenedione, a critical precursor for TT production. This study aimed to evaluate the frequency of polymorphisms in the *CYP17A1* and *HSD3B1* genes among PCa patients from Southwest Nigeria and to investigate their association with testosterone levels and androgen receptor (AR). The case-control study was conducted on 40 PCa patients and 40 control groups of healthy males with matching ages. Detection of *CYP17A1* and *HSD3B1* polymorphisms was done using TaqMan real-time polymerase chain reaction (RT-PCR), and estimation of TT and AR levels in serum was done using the enzyme-linked immune-sorbent assay (ELISA) technique for all groups. This study identified AA, AG, and GG genotypes of the CYP17A1 and AA and CA genotypes of HSD3B1, and there was no significant association between PCa and control groups of the genes. Testosterone levels were higher in the control group than in the PCa group (p=0.0015). There was no association was found between *CYP17A1* gene polymorphisms and TT and AR levels, similar to the *HSD3B1* gene and TT. Conversely, an association was found between the *HSD3B1* heterozygous adrenal-restrictive (CA) and AR, and no *HSD3B1* adrenal-permissive homozygous genotype (CC) was found. The result of this study suggests that the *HSD3B1* gene could be a suggestive prognostic and predictive biomarker of PCa. This would pave the way for future investigations that could influence diagnosis and personalised treatment of PCa.

Keywords: Prostate Cancer, Single nucleotide polymorphism, HSD3B1, CYP17A1, Testosterone