ENDOMETRIAL CARCINOMAS: MICROSATELLITE INSTABILITY AND SUSPECTED LYNCH SYNDROME IN THE GREATER JOHANNESBURG AREA (2009-2015)

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DECLARATION

I, Reubina Wadee declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the branch of Anatomical Pathology at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other University.

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Signature of candidate

The ____ day of _____ 2019

DEDICATION

I dedicate this thesis to my family: Nitesh, Caleesi and Cantara; but most of all to my Dad, Ahmed. Thank you to everyone for your love, patience and unwavering support of this endeavour. I could not have come this far without you.

PRESENTATIONS ARISING FROM THIS STUDY

I have presented posters based on my research at the following:

2017: Molecular Biosciences Research Thrust (MBRT) Research Day, University of the Witwatersrand, Faculty of Health Sciences and Faculty of Sciences, November 2017

2018: Faculty of Health Sciences Research Day, Faculty of Health Sciences, University of the Witwatersrand, September 2018

2018: The XXXII International Academy of Pathology (IAP) Congress, October 2018, Dead Sea, Jordan

2018: Molecular Biosciences Research Thrust (MBRT) Research Day, University of the Witwatersrand, Faculty of Health Sciences and Faculty of Sciences, November 2018

2019: United States and Canadian Academy of Pathology (USCAP) March 2019, National Harbour, Maryland, United States of America

PUBLICATION ARISING FROM THIS STUDY

"A potpourri of pathogenetic pathways in endometrial carcinomas with a focus on Lynch syndrome." In: Ann Diagn Pathol. 2019 Apr 1; 39:92–104. (Appendix 1).

ABSTRACT

Endometrial carcinomas are common malignancies of the female genital tract, with endometrioid endometrial carcinoma (EEC) being the most common histological subtype. Microsatellite instability is a molecular abnormality that is often documented in EEC and most tumours associated with Lynch syndrome (LS).

This study assessed 145 cases of EEC for the 4 mismatch repair markers by immunohistochemistry (IHC) and for microsatellite instability (MSI) by PCR. There were 41 cases that showed MMR deficiency, of which 37 demonstrated MLH1 loss. Forty-six cases were microsatellite unstable by PCR. The 37 MLH1 deficient cases and 25 cases illustrating discordance between IHC and PCR results underwent methylation studies, which revealed that over 80% of the 37 MHL1 deficient cases were hypermethylated. Furthermore, of the 25 cases showing discordant MMR IHC and MSI PCR results, 68% were hypermethylated. Of the remaining 8/25 cases, 7 were unmethylated whilst 1 case had insufficient DNA for methylation assessment.

BRAF assessment by IHC, PCR and Sanger sequencing was performed which showed that using all 3 tests; 6 out of 37 cases had BRAF mutations, which is higher than studies from western societies, but less than that noted in an eastern study. Similar to western studies, however, the present study showed that BRAF mutations are uncommon in EECs and should therefore not be included in the workup of EEC patients.

This study illustrated that a possible 13 of 145 (8.97%) patient cases are suspected of having potential germline mutations, which is double the expected frequency noted in the developed nations. This suggests that there may be a higher incidence of LS in South Africa than in western countries and highlights the need for screening tests in our patient population. It is

thus incumbent on histopathologists to undertake screening tests to identify females who may be affected by LS so that such patients, and their relatives; may be offered genetic counselling with a view to germline mutational assessment. Patients and relatives with suspected LS may then undergo surveillance for the development of other possible tumours in an attempt to decrease the menace of morbidity and mortality associated with this tumour syndrome.

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ABBREVIATIONS

°C	Degrees Celsius
µL	Microlitre
µm	Micrometer
A	Adenine
APC	Adenomatous Polyposis Coli
ARID	AT-rich interaction domain
bp	Base pair
C	Cytosine
CDH1	Gene encoding for epithelial cadherin
CDKN2A	Cyclin-dependent kinase Inhibitor 2A
CMMR-D	Constitutional Mismatch Repair Deficiency
Da	Dalton
Dkk	Dickkopf
EEC	Endometrioid endometrial carcinoma
EGFR	Epidermal growth factor receptor
EIC	Endometrial intraepithelial carcinoma
EMT	Epithelial to mesenchymal transitions
EPCAM	Epithelial cell adhesion molecule
ERBB2	Epidermal growth factor type II receptor
ERK	Extracellular signal-regulated kinase
FAP	Familial Adenomatous Polyposis
FBX7	F-Box and WD Repeat Domain Containing 7
FDA	Food and drug adminstration
FGF	Fibroblast growth factor
FGFR2	Fibroblast growth factor receptor 2
FZD	Frizzled
G	Guanine
GSK3β	Glycogen Synthase Kinase 3β
HNPCC	Hereditary Non-Polyposis Colorectal Carcinoma

IHC	Immunohistochemistry
InSiGHT	International Society for Gastrointestinal Hereditary Tumours
KRAS	Kirsten rat sarcoma
LEF/Tcf	T-cell factor/lymphoid enhancer factor
LS	Lynch syndrome
MALDI-TOF	Matrix-Assisted Laser Desorption/ionization Time Of Flight
MAP	Mitogen activated protein
MLH	Mut L homolog
MMR	Mismatch repair
MSH	Mut S homolog
MSI	Microsatellite instability
MSI-H(igh)	Microsatellite high
MSI-L(ow)	Microsatellite low
MSRE	Methylation Sensitive Restriction Enzymes
MSS	Microsatellite stable
mTor	Mammalian target of rapamycin
Mut	Mutational
NEEC	Non-endometrioid endometrial carcinoma
NTC	No template control
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase Chain Reaction
PI3K	Phosphatidylinositol 3-kinase
PIK3CA	p110α catalytic subunit of PI3K
PMS	Post-Meiotic Segregation proteins
POLE	DNA Polymerase Epsilon
PORTEC	Postoperative Radiation Therapy for Endometrial Carcinoma
PP2A	Protein phosphatase 2A
PPP2R1A	Protein Phosphatase 2 Scaffold Subunit Alpha
PROmISE	Proactive molecular risk classifier for Endometrial Cancer
PTEN	Phosphatase and tensin homolog
SOP	Standard Operating Procedure

SPRY	Sprouty mammalian genes
SWI/SNF	SWItch/sucrose non-fermenting
T	Thymidine
TCGA	The Cancer Genome Atlas
TP53	Tumour protein 53
U	Uracil
WNT	Wingless-type