

**MUTATION ANALYSIS USING MULTIPLEX LIGATION-
DEPENDENT PROBE AMPLIFICATION (MLPA) AND HIGH-
RESOLUTION MELTING CURVE (HRM) IN ISOLATED
HYPOSPADIAS PATIENTS**



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THESIS
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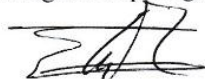
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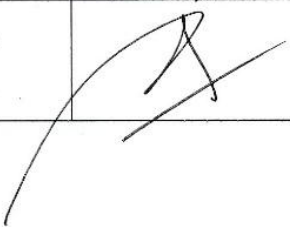
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CONTENTS

TITLE	i
APPROVAL SHEET	ii
REVISION APPROVAL SHEET	iii
DECLARATION	iv
CURRICULUM VITAE	v
ACKNOWLEDGEMENT	vi
CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
GLOSSARY	xiii
ABBREVIATIONS	xiv
LIST OF APPENDICES	xvi
ABSTRACT	xvii
ABSTRAK (BAHASA INDONESIA)	xviii
CHAPTER 1 INTRODUCTION	1
1.1 Background	1
1.2 Research Question	3
1.3 Research Purposes	3
1.4 Research Benefits	3
1.5 Originality	4
CHAPTER 2 LITERATURE REVIEW	6
2.1 Disorders of Sex Development	6
2.1.1 Definition of DSD	6
2.1.2 Phenotypes of 46, XY DSD	7
2.2 Hypospadias	8
2.3 Candidate Genes in Hypospadias	9
2.3.1 Genes involved in early development of the external genitalia	9
2.3.2 The defects in the late androgen-dependent phase.....	10

2.4 Endocrine Disruptors and the Gene-Environment Interaction	12
2.5 SHH and WNT5A as candidate genes for hypospadias	13
2.6 Genetic Counselling in Hypospadias Patients	16
CHAPTER 3 THEORETICAL AND CONCEPTUAL SCHEME	18
3.1 Theoretical Scheme	18
3.2 Conceptual Scheme	19
CHAPTER 3 RESEARCH METHODS	20
4.1 Research aspects	20
4.1.1 Field of study	20
4.1.2 Location of study	20
4.1.3 Period of study	20
4.1.4 Design of study	20
4.2 Material	21
4.2.1 Population	21
4.2.2 Samples	21
4.2.2.1 Inclusion Criteria	21
4.2.2.2 Exclusion Criteria.....	22
4.3 Laboratory Methods	22
4.3.1 DNA concentration.....	22
4.3.2 MLPA reactions	23
4.3.3 High-Resolution Melting Curve	24
4.3.4 Curve analysis	26
4.3.5 DNA sequencing	26
4.3.6 Mutation analysis	26
4.4 Variables	27
4.5 Data Analysis.....	27
4.6 Research Ethics	27
4.7 Research Flow.....	28
CHAPTER 5 RESULTS	29

5.1 MLPA with Hypospadias Candidate Genes as Probe Mix	29
5.2 Mutation Analysis with HRM	32
CHAPTER 6 DISCUSSION	39
CHAPTER 7 CONCLUSION AND FUTURE DIRECTIONS	44
CHAPTER 8 SUMMARY	45
REFERENCES	47
Appendix	53

LIST OF TABLES

Table 1 List of previous associated studies.....	4
Table 2 The thermal cycle of qPCR-HRM <i>SHH</i> exon 2	24
Table 3 The thermal cycle of qPCR-HRM <i>WNT5A</i> exon 2 and exon 5A	24
Table 4 The thermal cycle of qPCR-HRM <i>SHH WNT5A</i> exon 5B	24

LIST OF FIGURES

Figure 1 Genes involved in the development of male external genitalia	9
Figure 2 <i>SHH</i> gene	14
Figure 3 <i>WNT5A</i> gene	14
Figure 4 <i>WNT5A</i> signaling pathways.....	15
Figure 5 The summary of genes playing role in the normal development of male external genitalia.....	16
Figure 6 An Indonesian Patient with Hypospadias Probe Mix.....	29
Figure 7 Indonesian Patients with Hypospadias Probe Mix.....	30
Figure 8 Pakistani Patients with Hypospadias Probe Mix.....	30
Figure 9 Indonesian Patients with DSD and Hypospadias Probe Mix.....	30
Figure 10 Pakistani Patients with DSD and Hypospadias Probe Mix.....	31
Figure 11 Normalized melting curves	32
Figure 12 Melting peaks	32
Figure 13 Difference plot curve of samples with aberrant patterns.....	33
Figure 14 Sequencing result of sample PAK15 primer forward	35
Figure 15 Sequencing result of sample PAK15 primer reverse.....	35
Figure 16 Sequencing result of sample IDN4 primer forward.....	36
Figure 17 Sequencing result of sample IDN4 primer reverse.....	36
Figure 18 Unexpected finding on the <i>WNT5A</i> exon5B in 2 samples.....	39

GLOSSARY

Endocrine disrupting chemical (EDC)	: Chemical compounds which in certain doses might affect endocrine (or hormonal system) in mammals
CNV	: A form of structural variation as relatively large regions of DNA sequences as deletion or duplication
Hypospadias	: A congenital malformation with urethral opening not at the tip of the penis but along the anterior aspect of the penis
PCR	: A biochemical technique to amplify certain DNA sequence into thousand or million copy
HRM	: A biochemical technique to detect small differences in PCR melting (dissociation) curves
MLPA	: A variation of the multiplex polymerase chain reaction that permits multiple targets to be amplified with only a single primer pair
Sequencing	: A process to determine nucleotide sequence from given DNA fragments

ABBREVIATIONS

5DHT	: 5-Dihydrotestosterone
AR	: Androgen Receptor
ATF3	: Activating Transcription Factor-3
BMP	: Bone Morphogenetic Protein
CNV	: Copy Number Variation
CTGF	: Connective Tissue Growth Factor
CYP11A1	: Cytochrome P450, Family 11, Subfamily A, Polypeptide 1
CYR61	: Cysteine-rich angiogenic inducer 61
DEHP	: Diethylhexyl-phthalate
DHT	: Dihydrotestosterone
DSD	: Disorder of Sex Development
dsDNA	: Double-stranded Deoxyribonucleic Acid
EDC	: Endocrine Disrupting Chemical
EPHB	: Ephrin Beta
ESR	: Estrogen Receptor
FGF	: Fibroblast Growth Factor
FGFR	: Fibroblast Growth Factor Receptor
GADD45B	: Growth Arrest DNA Damage 45 Beta
GT	: Genital Tubercle
HOX	: Homeobox
HPLC	: High-performance liquid chromatography
HRM	: High-Resolution Melting
HSD17B3	: 17 β -hydroxysteroid Dehydrogenase 3
MAMLD1	: Mastermind-like Domain Containing 1
MgCl ₂	: Magnesium Chloride
MLPA	: Multiplex Ligation-dependent Probe Amplification

MMTV	:	Mouse Mammary Tumor Virus
mRNA	:	Messenger Ribonucleotide Acid
SHH	:	Sonic Hedgehog
SNP	:	Single Nucleotide Polymorphism
SRD5A2	:	Steroid 5 Alpha Reductase type 2
TGFB1	:	Transforming Growth Factor, Beta 1
TSP	:	Thrombospondin 1
WNT5A	:	Wingless-Type MMTV Integration Site Family, Member 5A
ZEB1	:	Zinc Finger E-Box Binding Homeobox 1

LIST OF APPENDICES

Appendix 1	Multiplex Ligation-dependent Probe Analysis.....	52
Appendix 2	High-Resolution Melting Primers	54
Appendix 3	High-Resolution Melting Results.....	55

ABSTRACT

Introduction: Hypospadias, one of the most common disorders of male genitalia is thought to have an increased trend in prevalence worldwide. Two important genes involved in the development of male external genitalia include *SHH* and *WNT5A*. Currently there was no study using multiplex ligation-dependent probe amplification (MLPA) and high-resolution melting curve (HRM) for hypospadias. The increased frequency in the last 20 years has been thought to be associated with environmental changes especially in the increased risk of exposure to chemical compounds. **Aim:** To identify mutations in hypospadias patients using MLPA and HRM. **Methods:** Samples are 17 Indonesian and 27 Pakistani DNA with a clinical diagnosis of hypospadias. MLPA and HRM were employed, continued with Sanger sequencing if aberrant patterns are found. **Results:** No abnormalities in CNVs were found using MLPA, and aberrant patterns were found using HRM in two samples and further analysis using Sanger sequencing resulted in translocations of 92 nt and 100 nt of chromosome 8 respectively. **Conclusion:** MLPA showed no abnormalities, while HRM showed aberrations. Translocations of chromosome 8 to *WNT5A* gene were found in two hypospadias patients.

KEYWORD: Hypospadias, MLPA, HRM, *SHH*, *WNT5A*

ABSTRAK

Pendahuluan: Hipospadia yang merupakan kelainan genitalia pria yang paling sering ditemukan diduga menunjukkan prevalensi yang semakin meningkat di seluruh dunia. Dua gen penting yang berperan dalam perkembangan genitalia eksternal pria antara lain adalah *SHH* and *WNT5A*. Hingga saat ini tidak ada penelitian yang menggunakan *multiplex ligation-dependent probe amplification* (MLPA) dan *high-resolution melting curve* (HRM) pada hipospadia. Meningkatnya frekuensi hipospadia dalam 20 tahun terakhir ini diduga terkait dengan perubahan lingkungan terutama risiko paparan terhadap zat kimiawi yang meningkat. **Tujuan:** untuk mengidentifikasi mutasi pada pasien dengan hipospadia dengan menggunakan MLPA dan HRM. **Metode:** Sampel adalah 17 DNA dari Indonesia dan 27 DNA dari Pakistan dengan diagnosis klinik hipospadia. Dilakukan analisis MLPA dan HRM yang dilanjutkan dengan *Sanger sequencing* jika terdapat pola abnormal pada HRM. **Hasil:** Tidak ditemukan kelainan CNV dengan menggunakan MLPA pada semua sampel, dan terdapat pola abnormal pada HRM di dua sampel. Analisis dengan *Sanger sequencing* menunjukkan translokasi sepanjang 92 nukleotida dan 100 nukleotida dari kromosom 8 secara berurutan. **Simpulan:** Tidak terdapat abnormalitas dari pemeriksaan MLPA namun pemeriksaan HRM menunjukkan adanya aberasi. Terdapat translokasi dari sebagian kromosom 8 ke dalam gen *WNT5A* pada dua pasien hipospadia.

Kata kunci: Hipospadia, MLPA, HRM, *SHH*, *WNT5A*