CHAPTER 1

INTRODUCTION

1.1. Background

Hypospadias, one of the most common disorders of male genitalia is thought to have an increased trend in prevalence worldwide. Hypospadias, a urethral tube defect in which the urethra opens ectopically on the ventral side of the penis, between the glans and the perineum, range from 7.84 to 33.8 per 10,000 births. This condition is included in the mild spectrum of 46, XY Disorder of Sex Development (DSD).[1–4] Hypospadias is the most common congenital penile anomaly (CPA) (68.3%), followed by chordee (8.6%) and hypospadias plus chordee (5%), and 14% are reported as unspecified penile anomalies. The severity of hypospadias can range from a slightly offset urethral meatus to complete failure of urethral tube formation, which can result in ambiguous genitalia.[5] 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.[2,3,6–8] The term isolated hypospadias is a technical term defining the patients not having syndromes and any other abnormalities.

Studies have been done to investigate the molecular mechanisms of external genitalia development of male individuals either in human or mice. The genes involved in the early phase of genitalia development can be excellent candidate genes in finding

the mutations in hypospadias.[9–13] While in the late androgen-dependent phase of the genitalia, *ATF3* and *ZEB1* are susceptible genes among others affected by exposure to estrogen from the environment known as endocrine disrupting compounds (EDC).[14–17]

The impact of EDC affecting the susceptible genes can be detected through expression and functional studies in exposed individuals. Thus genetic analysis is needed to understand these interactions. There is also a possible epigenetic involvement of the EDC in affecting the genes. The AR gene in target tissues from patients with hypospadias is more methylated than in control children.[18,19]

Factors playing roles in the development of CPA are genetics and environmental factors, particularly exposures to environmental endocrine disrupting chemicals (EDCs).[5] Two important genes involved in the development of male external genitalia include *SHH* and *WNT5A.* Those genes play roles in the early development that is androgen independent thus its examination is important. While the late development may involve more environmental factors and not merely genetic factors.

Currently there were limited studies using multiplex ligation-dependent probe amplification (MLPA) and high-resolution melting curve (HRM) for hypospadias. The study is aimed at understanding the molecular mechanism of gene-environment interaction by analyzing the mutations in the candidate genes from previous studies using MLPA and HRM. The findings will further contribute to the genetic counselling and screening of the susceptible genes in family.

1.2. Research Question

What are the mutations found using MLPA and HRM in patients with hypospadias patients?

1.3. Research Purposes

1.3.1 General Research Purposes

To identify mutations in hypospadias patients using MLPA and HRM.

1.3.2 Specific Research Purposes

1. To identify genetic variants (in particular copy number variants or CNVs) in hypospadias candidate genes in hypospadias patients by using MLPA technique.
2. To identify genetic variants in *SHH* and *WNT5A* genes in hypospadias patients using HRM technique.

1.4 Research Benefits

The benefits of this study are:

1. To learn and develop the MLPA technique for screening of hypospadias candidate genes in hypospadias patients.
2. To learn and develop the high resolution melting technique for screening of *SHH* and *WNT5A* genes in hypospadias patients.

3. To provide a more detailed genetic counseling and screening practice for family by understanding the gene-environment interaction and complex etiology in hypospadias.

1.5 Originality

This is the first study combining MLPA and HRM for screening of candidate genes in hypospadias patients. Some previous researches are listed below.

Table 1. List of previous associated studies

No	Author	Title of Publications	Method	Result
1.	Kalfa N, Liu B, Klein O, Wang MH, Cao M, Baskin LS.	Genomic variants of *ATF3* in patients with hypospadias. J Urol 2008; 180:2183-8; discussion 2188.	Direct sequencing of coding exons and splice sites of *ATF3* was performed in 41 boys with hypospadias and 30 controls. In addition, *ATF3* expression in 1 human fetal penis with and 1 without hypospadias was studied by immunohistochemical analysis.	A missense variant was identified in a boy with anterior hypospadias. hTree genomic variants were found in or close to exon 6 in patients with perineal, penoscrotal and anterior hypospadias.
2.	Beleza-Meireles A, Lundberg F, Lagerstedt K, et al.	*FGFR2, FGF8, FGF10* and *BMP7* as candidate genes for hypospadias. Eur J Hum Genet 2007;15:405-10.	DNA from 60 boys with familial, isolated, hypospadias was screened for mutations in *FGFR2*, *FGF10*, *FGF8*, and *BMP7* genes, using DHPLC and DNA sequence analysis.	The sequence variations c.590C>G and c.582-62G>A in *FGF8*, and, c.550+27C>T, c.727+180T>G, c.830T>C, c.2454C>T in *FGFR2* were found uniquely in

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				hypospadias patients, as compared with 96 controls.
3.	van der Zanden LF, van Rooij IA, Feitz WF, et al.	Genetics of hypospadias: are single-nucleotide polymorphisms in *SRD5A2*, *ESR1*, *ESR2*, and *ATF3* really associated with the malformation? J Clin End Metab 2010 ;95:2384-90.	Genotyping of 620 Caucasian hypospadias cases and 596 controls for these SNPs using TaqMan-based genotyping.	The SNPs in *ESR2* and *ATF3* were borderline associated with hypospadias [odds ratios 0.9 (95% confidence interval 0.7-1.0) and 1.2 (95% confidence interval 1.0-1.4)
4.	Chen Y, Thai HT, Lundin J, et al.	Mutational study of the *MAMLD1*-gene in hypospadias. Eur J Med Genet 2010;53:122-6.	Direct sequencing of the *MAMLD1* gene in 99 sporadic hypospadias cases	Five non-synonymous mutations, one synonymous and one non-coding mutation were found.