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Association of MAMLD1 single-nucleotide polymorphisms with hypospadias in Chinese Han population

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1. ABSTRACT

Hypospadias is one of the most common congenital malformations among children. Both gene mutations and environmental factors are thought to be involved in the development of hypospadias. The mastermind-like domain-containing 1 gene (*MAMLD1*, formerly *CXorf6*) is a new candidate gene and its mutation has been shown in some cases of hypospadias. Here, by direct sequencing of PCR products, we assessed and found mutations that occur in 220 sporadic cases of hypospadias. The mutations p.N589S (c.1766A>G) was found at a significantly higher rate among patients with hypospadias.

2. INTRODUCION

Hypospadias is one of the most common congenital defects in humans and occur in about 0.04% of living births in China (1), which is multifactorial and due to both genetic and environmental factors. This condition is characterized by displaced urethral orifices along the ventral side of the penis. The development of the external genitalia in males is a complex process influenced by multiple genes including *SRD5A2* a *5A-REDUCTASE* and androgen receptor (*AR*) (2). However, in majority of cases, the etiology of hypospadias remains unknown (3).

Recently mutations in Mastermind-like domain-containing 1 gene (*MAMLD1*, formerly *CXorf6*) have been reported and it is thought that such mutations are involved in the development of hypospadias. *MAMLD1* maps to proximal Xq28, was discovered in the course of identifying the gene (*MTM1*) responsible for myotublar myopathy (4-6). *MAMLD1* is expressed in the gonads during sex differentiation and interacts with steroidogenic factor (*SF-1*), a regulator of the transcription of genes involved in testicular differentiation. *MAMLD1* mutations may impair or interfere with androgen metabolism (10-12). Newborns with microdeletions of *MTM1* that extend to the *CXorf6* locus not only exhibit myopathy, they show external genital malformations. Analsysis of *MAMLD1* has identified several mutations: Fukami *et al.* identified three non-sense mutations (p.E124X, p.Q197X and p.R653X) in 4 individuals (46XY, DSD) with hypospadias. These were associated with micropenis, bifid scrotum and penoscrotal hypospadias (7). Among other mutations were Q529K and p.D686D and 1295T/C (V432A), as well as p.531ins3Q and 325delG which were associated with proximal hypospadias (8-9).

To decipher the true incidence of genetic mutations in hypospadias, we investigated the mutations which occur in *MAMLD1* gene in a large number of sporadic Chinese hypospadia cases and a control male group of subjects.

3. MATERIALS AND METHODS

3.1. Patients and controls

Two hundred healthy male controls and two hundred and twenty Chinese (Han) individuals (from 0.5 to 8 years of age) with the genotype of 46XY who were surgically treated for hypospadias were recruited for the study. Clinical phenotypes and complications

Table 1. Clinical data of 220 patients with hypospadias

Type of hypospadias	Glandular	Penile	Penoscrotal	Scrotal	Perineal
No. of cases	48	63	39	41	29
Average age (years)	3.9	4.1	5.2	5.9	6.6
Complications	3	5	8	6	8
Micropenis[1]	0	0	4	5	6
Cryptorchidism	1	3	3	1	1
Hernia	2	2	1	0	1

[1]Micropenis: length of penis is smaller than the average penile length in males of matched age.

Table 2. Genetic variants detected by sequencing of the *MAMLD1* gene in cases and controls

Nucleotide change(Amino acid change)	rs number	Number of cases with hypospadias	Number of controls
c.1766A>G (p.N589S)	rs2073043[1]	15	6

Total cases 220. [1]Previously described (7,9)

Table 3. Genotype and phenotype of patients with mutations

Type of Hypospadias	Other abnormal phenotypes
Penile	None
Penile	Micropenis[1]
Penoscrotal	Micropenis[1]
Penoscrotal	Bifid scrotum
Scrotal	Ventricular and atrial septal defects
Penile	None
Glanular	None
Penile	None
Penile	None
Penile	None
Penile	None
Glanular	None
Penile	None
Glanular	None
Penile	None

[1]Micropenis: length of penis is smaller than the average penile length in males of matched age.

of the patients are listed in Table 1 and ranged from perineal to cleaved prepuce. The Ethics Committee at Renji hospital approved the study and informed consents were obtained from parents.

3.2. DNA extraction

DNA was extracted, using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), from peripheral blood or foreskin. DNA concentration was measured by UV photometer (Eppendorf, Hilden, Germany).

3.3. PCR amplification and sequencing

Direct sequencing was performed on the coding exons 3-6 and their flanking intronic sequences (~100 bp on each side) of the *MAMLD1*. Primers used for amplification were previously described (13). 100-200 ng of genomic DNA, 5 μL of 10×Ex Taq Buffer, 4 μL of dNTP Mixture (2.5.mM each), 0.4 μM of each primer, and 2.5 units of Ex Taq DNA polymerase (Takara, Japan) were mixed and subjected to polymerase chain reaction (PCR) using Mastercycler Thermal Cycler (Eppendorf). Amplified PCR products were purified from agarose gel using QIAquick Gel Extraction Kit (Qiagen) and sequenced by AB13130XL sequencer (Applied Biosystems, USA).

3.4. Statistical analysis

Haplotype frequencies between cases and controls were compared using the Chi square test and Fisher test using software SPSS 16.0. Hapmap and ensembl.org were used to exclude linkage

Table 4. Genetyping data in cases and controls for p.N589S polymorphisms

Polymorphism	Alleles		Experimental group		Control group		P
	Nucleotide	Amino acid	Cases	Ratio	Cases	Ratio	
p.N589S	A	N	205	0.93	194	0.97	0.06>0.05
	G	S	15	0.07	6	0.03	

disequilibrium. The results are expressed as means ±SD, and statistical significance was assessed by the t-test.

5. RESULTS AND DISCUSSION

In 286 Asians, others have reported 3 cases of SNP mutation and among these, the polymorphism, p.N589S has been previuolsy reported as a low risk factor in the development of hypospadias (7, 9). We were unable to replicate the results of three earlier studies in Caucasian and non-Caucasian populations perhaps due to population specificity in the detection of mutation or differences in the criteria used to select cases and/or controls (10). To minimize the chance of population stratification, we chose Chinese Han cases. There were no statistical significance between mutation rates in the control group as compared with those with hypospadias who exhibited p.P589S polymorphism (Table 4) and these mutations did not show association with hypospadias (p >0.05). While p.N589S (c.1766A>G) polymorphism was present in 6/200 controls, it was found in 15/220 in patients with hypospadias (6.8% vs. 3%, p >0.05).

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Yidong Liu and Likai Zhuang are both first authors.

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