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Original Research Article

Are anti-Müllerian hormone and its receptor polymorphism associated with the hormonal condition of undescended testes?

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ABSTRACT

Purpose: Numerous genetic and endocrine factors are involved in the process of testicular descent, but only a few genetic causes have been reported in human. The aim of this study was to investigate the density and distribution of single nucleotide polymorphisms (SNPs) anti-Müllerian hormone (AMH) and AMHRII receptors in cryptorchid patients and determine potential hormone imbalance connected with undescended testes by assessing the levels of AMH, Insulin-like factor 3 (INSL3) and inhibin B.

Materials and methods: The serum hormone levels (AMH, INSL3 and inhibin B) were compared in the two groups – cryptorchidism ($n = 105$) and control group ($n = 58$). The frequency of AMHRII −482 A > G, AMHRII IVS 10 + 77 A > G, AMHRII IVS 5–6 C > T, and AMH Ile49Ser polymorphisms among cryptorchid boys were compared with the control group.

Results: None of the hormones levels were different between the cryptorchid and the control groups. All cases of IVS 5–6 C > T homozygote and heterozygote mutation were accompanied by an IVS 10 + 77 A > G and 482 A > G homozygote and heterozygote mutation. Interestingly, in most cases of all four polymorphisms, homozygote recessive genotype was associated with cases of cryptorchidism. However, the groups of patients were too small to draw definite conclusions.

Conclusion: The AMHRII −482 A > G, AMHRII IVS 10 + 77 A > G, AMHRII IVS 5–6 C > T and AMH Ile49Ser genotypes should be determined in a much larger group of boys with cryptorchidism.

1. Introduction

The etiology of undescended testis is complex. Numerous genetic and endocrine factors are involved in the process of testicular descent [1]. Most cases remain idiopathic. The current model of testicular descent involves testicular differentiation from the ambisexual gonad in the presence of the Y chromosome, with production of anti-Müllerian hormone (AMH), from the developing Sertoli cells, and production of testosterone and Insulin-like factor 3 (INSL3) from the Leydig cells. Previously, male gonads were usually considered to be quiescent organs in infancy and childhood until puberty. Now we know that is not true. Recent studies suggested that the early postnatal period could be a crucial and critical stage of germ cell development [2]. The physiological two-stage testicular descent process [3] is dependent on the proper functioning of the hypothalamus–pituitary–testicular axis and a proper androgen and estrogen balance [4]. The first transabdominal phase is dependent on Insulin-like peptide 3 (Insl3), which mediates gubernaculum enlargement. The second inguino-scrotal phase is regulated by androgen. In fetal life, AMH is secreted by the Sertoli cells, where it induces regression of the Müllerian ducts. A recent study demonstrated that genetic variants in the AMH and AMH type II receptor (AMHRII) genes may be associated with female infertility [5]. According to Schuh-Huerta et al., single nucleotide polymorphisms (SNPs) of AMH genes may lead to female infertility, because they affect both the follicle counts and folliculogenesis [6]. There are no such studies on male infertility.

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The aim of this study was to determine hormone imbalance connected with undescended testes by assessing the levels of AMH, INSL3, and inhibin B. We also investigated the density and distribution of AMH and AMHRII SNPs receptors in cryptorchid patients. Additionally, we wanted to investigate the correlation between the hormonal balance of the testes and single nucleotide polymorphisms of AMH and AMH II receptors. This is the first study to explore the distribution of AMH RII single nucleotide polymorphisms in the Polish population of cryptorchid boys. This study will also help elucidate whether AMH and AMH RII SNPs can be applied as genetic markers in the prediction of further male infertility in cryptorchid boys.

2. Materials and methods

The study involved 105 boys with unilateral cryptorchidism (1–4 years). All boys suffered from congenital primary cryptorchidism. The control group consisted of 58 boys with inguinal hernia, of the same age range. All participating boys were the patients of the Department of Pediatric Surgery, Medical University of Bialystok (Poland). In the morning of the day of surgery, between 6 and 7 am, a 5 ml sample of blood was taken from all patients. We analyzed the value of AMH, INSL3, inhibin B and single nucleotide polymorphisms in AMH and AMH II receptors.

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the local Medical Ethics Committee (Approval No. R-I-002/485/2012). Hormone measurements and genotyping were made after written informed consent was obtained from the caregivers of the patients.

2.1. Hormone level measurements

Serum samples from the patients and controls were kept at −80°C until the hormone analysis. The level of INSL-3, AMH and inhibin B were assessed using commercial enzyme-linked immunosorbent assay ELISA kits Beckman Coulter and Uscn Life Science Inc.

For determination of the levels of INSL-3, AMH and inhibin B, we used plates (each consisting 96 of holes), coated with monoclonal antibodies specific to INSL-3, AMH and inhibin B. All samples were pipetted into the wells and INSL-3, AMH and inhibin B were bound by immobilized antibody. The plates were incubated and washed several times. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for INSL-3, AMH and inhibin B were added to the wells, and the samples were reincubated. Next, a substrate containing H_2O_2 and tetramethylbenzidine was added to the reaction wells and the color developed proportionally to the amount of cytokines. The color development was stopped by adding 1 M H_2SO_4. Staining intensity was measured spectrophotometrically using ELISA (ANTHOS, Australia) microplate reader at 450 nm, with the correction wavelength set at 620 nm. The results were calculated based on the linear (points to points) standard curve and expressed.

The minimum detectable amount was: 0.16 ng/ml for AMH, 0.14 pg/ml for inhibin B, and 6.1 pg/ml for INSL-3.

2.2. AMHRII and AMH genotyping

Genomic DNA was extracted from 200 μL of frozen whole blood samples using QIAamp DNA Mini Kit (Qiagen, Poland) according to the manufacturer's instructions. The *AMHRII* −482 A > G (rs2002555), *AMHRII* IVS 10 + 77 A > G (rs11170555), *AMHRII* IVS 5–6 C > T (rs2071558) and *AMH* Ile49Ser (rs10407022) genotypes were determined using a commercial kit for allelic discrimination – TaqMan SNP Genotyping Assays (Life Technologies, Poland). We used the Assay IDs: C___1673084_10, C__25471905_10, C___1673083_1_ and C__25599842_10 according to the manufacturer's standard protocols.

The TaqMan® genotyping assay was performed in the *ABI Prism 7900HT Sequence Detection* System (Life Technologies, Poland). Genotypes were estimated by investigators blinded to the patient's clinical data. To control for correct handling procedure, genotyping was repeated twice. Both procedures revealed identical outcomes.

Table 1
Mean values (±SD) of INSL3, AMH and inhibin B in boys with cryptorchidism and the control group.

Hormones	Cryptorchid $n = 105$	Control group $n = 58$	p value
INSL3 (pg/ml)	2672.000 (±1334.4)	3267.500 (±1505.9)	=0.05
AMH (ng/ml)	118.200 ± 59.996	111.500 (±59.5)	>0.05 (=0.93)
Inhibin B (pg/ml)	113.770 ± 54.241	129.575 (±78.646)	>0.05 (=0.08)

2.3. Statistical analysis

Statistical analyses were carried out using Statistica 10.0 StatSoft. The Mann–Whitney *U* test was used to compare the groups. p values of less than 0.05 were considered significant.

3. Results

The serum hormone levels (AMH, INSL3 and inhibin B) were compared in the two groups (Table 1). None of the hormones levels were different between the cryptorchid and the control groups. INSL3 in the cryptorchid and the control groups were 2672.000 ± 1334.407 pg/ml and 3267.500 ± 1505.904 pg/ml, respectively ($p = 0.05$); AMH 118.200 ± 59.996 ng/ml and 111.500 ± 59.518 ng/ml, respectively ($p = 0.93$) and inhibin B 113.770 ± 54.241 pg/ml and 129.575 ± 78.646 pg/ml, respectively ($p = 0.08$).

The frequency of *AMHRII* −482 A > G (rs2002555), *AMHRII* IVS 10 + 77 A > G (rs11170555), *AMHRII* IVS 5–6 C > T (rs2071558), and *AMH* Ile49Ser (rs10407022) polymorphisms among cryptorchid boys were compared with the control group. The results are shown in Fig. 1.

All cases of IVS 5–6 C > T homozygote and heterozygote mutation were accompanied by an IVS 10 + 77 A > G and −482 A > G homozygote and heterozygote mutation.

Association of AMH and AMHRII receptor polymorphism with AMH, INSL3 and inhibin B serum levels did not show statistical significance.

Interestingly, in most cases of all four polymorphisms, homozygote recessive genotype was associated with cases of cryptorchidism. However, the groups of patients were too small to draw definite conclusions. The *AMHRII* −482 A > G, *AMHRII* IVS 10 + 77 A > G, *AMHRII* IVS 5–6 C > T and *AMH* Ile49Ser genotypes should be determined in a much larger group of boys with cryptorchidism.

4. Discussion

No previous studies have examined the possible association between AMH and AMHRII receptor single nucleotide polymorphisms (SNPs) and levels of serum INSL3, inhibin B and AMH in cryptorchidism. Although we did not find any significant association, interestingly all cases of IVS 5–6 C > T homozygote and heterozygote mutation were accompanied by IVS 10 + 77 A > G