ADOLESCENTS & YOUNG ADULT MEN WITH HYPOSPADIAS:

TESTICULAR FUNCTION AND IN-DEPTH GENETIC SCREENING

L Tack, AF Spinoit, P Hoebeke, S Riedl, A Springer, U Tonnhofer, M Hiess, J Weninger, A Mahmoud, K Tilleman, E Van Laecke, A Juul, J Albrethsen, E De Baere, H Verdin and M Cools

MEDICAL UNIVERSITY OF VIENNA

UZ GENT UNIVERSITEIT GENT

KEY MESSAGES

- Hormone levels were reassuring
- Oligo- /azoospermia:
  - 30% of AGA cases with complex hypospadias
  - 60% of SGA cases
- No pathogenic monogenic variants or oligogenic variant combinations found

INTRODUCTION

Background: Hypospadias affects approximately 1/200 newborn males and is sometimes included in the DSD spectrum. Few data currently support this hypothesis.

Aims: To assess endocrine and reproductive outcome of young men born with hypospadias and explore genetic mechanisms underlying the development of hypospadias and eventual testicular dysfunction.

METHODOLOGY

Study design: Cross-sectional study (Ghent and Vienna University Hospitals)

Participants: Young adult men (16-21 years) born with non-syndromic hypospadias (n=193) and healthy male controls (n=50)

Assessments: Physical exam, hormonal status (gonadotropins, androgens, insulin-like factor 3 (INSL3) & inhibin B), semen analysis and molecular genetic analysis (NGS-based panel of 474 genes & Oligogenic Resource for Variant AnaLysis (ORVAL))

PARTICIPANTS

	Appropriate for gestational age (AGA)	Small for gestational age (SGA)
Distal hypospadias	122/167 (73.1%)	10/26 (38.5%)
Midshaft hypospadias	33/167 (19.8%)	5/26 (19.2%)
Proximal hypospadias	12/167 (7.2%)	11/26 (42.3%)
Complex hypospadias	16/167 (9.6%)	7/26 (26.9%)
Controls	n=50	

RESULTS

Physical exam & hormone assays

Physical exam:

- Smaller stretched penile length in SGA cases
  - Regardless of number of penile surgeries and height
- All had a normal timing and pace of puberty

Gonadotropin levels:

- Gonadotropin & INSL3 levels were higher in cases compared to controls
  - Few above laboratory thresholds
- Few cases had androgen levels below the laboratory thresholds
  - All with isolated hypospadias
  - And in absence of elevated gonadotropins

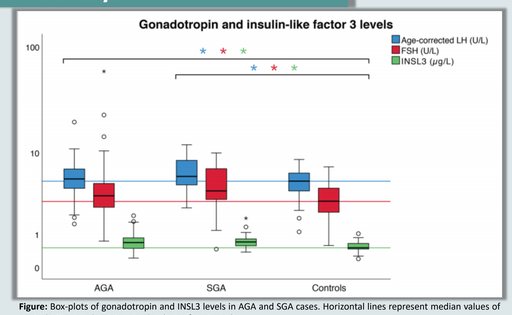


Figure: Box-plots of gonadotropin and INSL3 levels in AGA and SGA cases. Horizontal lines represent median values of controls. Y-axis in logaritmic scale. *:significant result.

Genetics

- No (likely) pathogenic monogenic variants (according to ACMG criteria)
- No digenic variant combinations (predicted ≥95%-likely disease-causing) were withheld

AGA cases

	Phenotype	Gene	Segregation analysis	HGVS transcript	HGVS Protein	rsID	REVEL score	GnomAD VAF	GnomAD HMZ
Case 1	M, Cb	BNC2	De novo	c.1022A>G	p.(Asn341Ser)	-	0,156	-	-
Case 2	M	CHD7	Paternal/de novo+	c.3730A>G	p.(Thr1244Ala)	-	0,722	-	-
		NR5A1	Paternal/de novo+	c.629C>T	p.(Pro210Leu)	rs900214501	0,18	0.000006569	0
Case 3	D	ESR2	Maternal	c.1123G>C	p.(Glu375Gln)	-	0,866	-	-
Case 4	D	ZFPM2	NA	c.89A>G	p.(Glu30Gly)	rs121908601	0,329	0,002970	2
Case 5	D, Mic, B	DGKK	NA	c.255_290dl	p.(Ser91_Ala102del)	-	-	0,0009738	26*
Case 6	M	GATA4	NA	c.1037C>T	p.(Ala346Val)	rs115372595	0,592	0,001432	0
Case 7	P	LHCGR	NA	c.1046C>T	p.(Ala349Val)	rs758729322	0,596	0.000006572	0
Case 8	P	ERBB4	NA	c.2444T>C	p.(Ile815Thr)	rs1264168721	0,756	0.00001314	0

SGA cases

	Phenotype	Gene	Segregation analysis	HGVS transcript	HGVS Protein	rsID	REVEL score	GnomAD VAF	GnomAD HMZ
Case 9	M	BNC2	Maternal	c.2618C>T	p.(Pro873Leu)	-	0,494	-	-
Case 10	P	ESR2	NA	c.64A>G	p.(Ile22Val)	rs76299711	0,269	0.0006192	0
Case 11	P, B	LHCGR	NA	c.1435C>T	p.(Arg479Ter)	rs757225917	-	0.000003981	0
Case 12	P, Cb	NR5A1	NA	c.374C>T	p.(Pro125Leu)	rs780952265	0,24	0.00002207	0
Case 13	D	ZNRF3	NA	c.925T>A	p.(Ser309Thr)	rs769697204	0,09	0.00001227	1
Case 14	P	LHCGR	NA	c.1847C>T	p.(Ser616Phe)	-	0,866	-	-
Case 15	D	EP300	NA	c.5869C>T	p.(Pro1957Ser)	rs1301322622	0,217	0.00001415	0
		ESR2	NA	c.661A>G	p.(Arg221Gly)	rs78851986	0.794	0.002047	0

HMZ: homozygotes; M: Midshaft hypospadias, Cb: bilateral cryptorchidism, D: distal hypospadias, Mic: micropenis, B: bifid scrotum, P: proximal hypospadias; REVEL score: rare exome variant ensemble learner; VAF: variant allel frequency; Homozygotes: reported number of homozygotes or hemizygotes (*); +: No DNA was available of the deceased father (who had hypospadias at birth). GnomAD v3.1.1.

Semen analysis

Oligo-/azoospermia:

- 18.6% of hypospadias cases *vs* 4% of controls
- 59.1% of SGA cases
- 31.3% of AGA cases with complex hypospadias

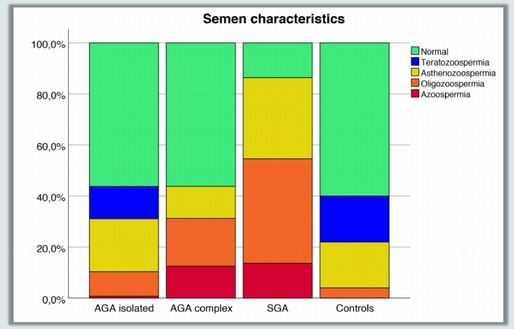
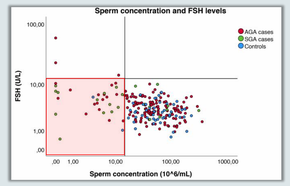
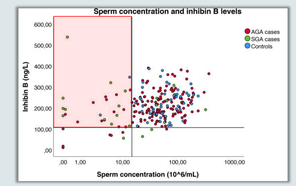


Figure: Summary of semen characteristics. Azoospermia: no sperm cells found on two separate samples; Oligozoospermia: <15.106/mL; Asthenozoospermia: normal concentration and progressive motility <32% and total motility <40%; Teratozoospermia: normal concentration, motility and morphology <4%.

Hormone levels as predictors of oligo- / azoospermia:

- FSH: sensitivity 9.4%, specificity 100%
- Inhibin B: sensitivity 21.9%, specificity 97.9%
- ROC curve analysis: criterion FSH 4.11U/L ; Inhibin B 196.4ng/L
- Higher INSL3 levels (p=0.042)





Figures: Left: Scatterplot of sperm concentration and FSH levels. Horizontal line: 15.106/mL; vertical line: laboratory threshold of 12.4U/L. X and Y-axis in logarithmic scale. Right: Scatterplot of sperm concentration and inhibin B levels. Horizontal line: 15.106/mL; vertical line: laboratory threshold of 105 ng/L. X and Y-axis in logarithmic scale. Red boxes represent those with reduced sperm concentration and hormone levels within laboratory reference values.

CONCLUSION

- All participants had a normal timing and pace of puberty
  - Mainly subclinical hormone abnormalities
- Semen quality is a major concern in hypospadias, especially in SGA and complex hypospadias cases
- No recurrent genetic causes were identified, no arguments for oligogenic effects
  - Non-coding variants? Epigenetics? Placental insufficiency? Multifactorial?

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Ghent University Hospital, Pediatric Endocrinology & Diabetology (3K12D), BELGIUM, Contact: Martine.Cools@ugent.be