Polymorphisms and mutations of the genes INSL3 and HOXD13 in the pathogenesis of isolated cryptorchidism in Greece

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Abstract

Current literature suggests an important role of both endocrine disruptors and genetic factors in the occurrence of cryptorchidism. The aim of the study is to investigate the impact of variants in INSL3 and HOXD13 genes in the pathogenesis of isolated cryptorchidism in Greece. Forty-three boys with isolated cryptorchidism and 50 healthy non-cryptorchidic boys (control group) were enrolled. Genomic DNA was extracted from peripheral blood leukocytes and genetic analysis was conducted using PCR and direct sequencing of Insl3 and HOXD13 gene regions. Two apparently novel variants, the * -109 T>A of the Insl3 5' UTR and the *528_529inv of the HOXD13 3' UTR were disclosed in two unrelated patients. None of these variants was revealed in the control group (p=0.32304). Conversely, multiple previously described polymorphisms of both genes (Insl3: c.27G>A, c.126A>G and c.178A>G/ HOXD13: c.*311C> T, c.*360A> T and c.*359_*360insT) were detected in both the cryptorchidic patients and the control group with no statistically significant difference between groups. "In silico" analysis for the two as yet unreported findings indicated possible alterations of the cDNA sequences but with no comprehensible impact on the coding procedure. A combination of polymorphic alleles in these two genes was observed in both patients and controls without any statistically significant difference between groups (p=0.30873). Neither the presence of specific polymorphisms in the INSL3 and HOXD13 genes, nor their combination could account for the pathogenesis of isolated cryptorchidism. The effect of endocrine disruptors or variations in non-examined genes in the pathogenesis of cryptorchidism, as well as the better delineation of the role of the new detected variants should be further investigated in larger populations.

Objectives

The aim of the present study was to investigate the impact of mutations or polymorphisms of the genes INSL3 and HOXD13 in the pathogenesis of isolated cryptorchidism in Greece, since cryptorchidism occurrence demonstrates a specific different geographic distribution.

Patients and Methods

A total of 43 patients with a mean age ±SD of 3 ±2,6 years were selected from the Pediatric Surgery Clinics of "Agia Sofia" and "Aglaia Kyriakou" Children's Hospitals. A total of 50 non-cryptorchid boys with a mean age ±SD of 7± 3,68 years were selected as control group from the Otorhinolaryngological (ENT) Clinic of "Agia Sofia" Children's Hospital. The audit which was performed in all samples included polymerase chain reaction to amplify the desired sequence, enzymatic mismatch analysis (ECMA) for probable heterozygous change-mutation/polymorphism detection and analysis of the primary structure of DNA in those samples that presented some evidence by ECMA.

Table1: Subjects' characteristics

	Patients	Controls	p-value
Number	43	50	ns
Age (mean ± SD)	3± 2.6	7 ±3.68	p = 4.35145523752E-6
Spontaneous conception/IVF	40/3	47/3	p = 0.85
Unilateral cryptorchidism	32	n/a	n/a
Bilateral cryptorchidism	11	n/a	n/a
Gestational week	38.45±2.06	37.79±2.11	p = 0.14
Birth weight	3.1±0.61	3.12±0.55	p = 0.99
Family history of cryptorchidism	9/43	5/50	p = 0.15
Vaginal delivery/ caesarean delivery	21/22	26/24	p = 0.76

Results

Two unknown variants were identified, the variant * 1-109 T> A in the INSL3 gene and the unknown * 528 _529inv in the HOXD13 gene in cryptorchidic patients and both were not detected in any of the controls. Many known variants in genes INSL3 and HOXD13 were identified both in patients and controls with no statistical difference between the two groups. Moreover, some of the cryptorchidic patients and some of the controls demonstrated a combination of polymorphic alleles, affecting both the INSL3 and the HOXD13 genes, again with no statistically significant difference between the two groups. High frequency of two polymorphisms, c. 126 A>G and c. 178 A>G in INSL3 gene was revealed in Greece in the homozygous or heterozygous state with significantly higher frequency among controls, proving that they could not have a role in the pathogenesis of cryptorchidism.

Table 2.1 Results

Genes	Region	Patients	Frequency (%)	Controls	Frequency (%)	P-value
INSL3						
Exon 1						
***-109 T>A (heterozygous)**	**5' UTR**	**1/43**	**2,3**	**0/47**	**0**	**0.323**
27 G>A (heterozygous)	exon 1	2/43	4,7	3/47	6,4	0.722
27G>A(homozygous)	exon 1	0/43	0	1/47	2,1	0.322
27G>A (homo + hete)	exon 1	2/43	4,7	4/47	8,5	0.464
126 A>G (heterozygous)	exon 1	5/43	11,6	8/47	17	0.470
126 A>G (homozygous)	exon 1	0/43	0	10/47	21,3	0.001
126 A>G (homo + hete)	exon 1	5/43	11,6	18/47	38,3	0.003
178 A>G (homozygous)	exon 1	24/43	55,8	21/46	45,7	0.344
178 A>G (heterozygous)	exon 1	1/43	2,3	10/46	21,74	0.004
178 A>G (homo + hete)	exon 1	25/43	58,1	31/46	67,44	0.373
HOXD13						
Exon 2						
*311 C>T (heterozygous)	3'UTR	5/43	11,6	6/50	12	0.956
*311 C>T (homozygous)	3'UTR	0/43	0	1/50	2	0.322
*311 C>T (homo + hete)	3'UTR	5/43	11,6	7/50	14	0.736
*359_*360insT	3'UTR	1/43	2,3	0/50	0	0.323
*360 A>T (heterozygous)	3'UTR	1/43	2,3	0/50	0	0.323
***528 _529 inversion**	**3'UTR**	**1/43**	**2,3**	**0/50**	**0**	**0.323**

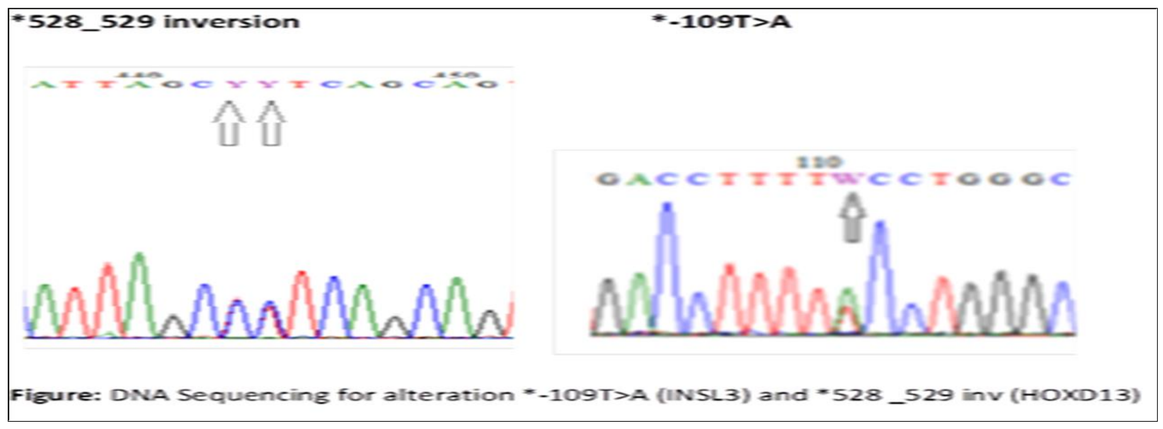

Figure: DNA Sequencing for alteration *-109T>A (INSL3) and *528 _529 inv (HOXD13)

Table 2.2 Combined Polymorphisms

Defects	Patients	Frequency	Controls	Frequency	p-value
Combine molecular defects of different genes	6/29	20%	4/36	11%	0.309
Combine molecular defects of the same gene	6/29	20%	18/36	50%	0.012

Conclusions

We could not identify the presence of specific polymorphisms in the INSL3 and HOXD13 genes, nor their combination as the underlying cause in the pathogenesis of isolated cryptorchidism. Further investigations in larger populations should be conducted in order to better characterize the impact of non-examined genes or of endocrine disruptors, as well as the better delineation of the role of the new detected variants, in the pathogenesis of cryptorchidism.

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