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Genetic Alterations Associated With Cryptorchidism

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CRYPTORCHIDISM IS THE FAILURE of one or both testes to descend into the scrotal sac and is the most frequent congenital birth defect in male children (2%-4% in full-term male births).¹ Although cryptorchidism is often considered a mild malformation, it can seriously affect men's health, representing the best characterized risk factor for infertility and testicular cancer in adulthood.² Important advances in the comprehension of the physiology of testicular descent during fetal life and in the clinical management of boys with cryptorchidism have been achieved in the last few years, but the etiology of cryptorchidism remains mostly unknown.^{2,3}

Recent studies have elucidated that the major regulators of testicular descent are the Leydig cell-derived hormones insulin-like factor 3 (INSL3) and testosterone, involved, respectively, in the transabdominal and inguinoscrotal phase of testicular descent² and acting on their respective receptors, RXFP2 (relaxin family peptide 2) and AR (androgen receptor). A better knowledge of testicular descent and the identification of animal models of cryptorchidism⁴ has facilitated research on the possible genetic contribution to human cryptorchidism. Genetic causes might be related to chromosomal alterations or mutations in *INSL3*/

Context Cryptorchidism is the most frequent congenital birth defect in male children and represents an important risk factor for infertility and testicular cancer. Major regulators of testicular descent are the hormones insulin-like factor 3 (INSL3) and testosterone, and disruption of these pathways might cause cryptorchidism.

Objective To determine the frequency of genetic alterations in cryptorchidism.

Design and Setting Case-control study in 2 departments of pediatric surgery in Italy between January 2003 and March 2005.

Patients Six hundred male infants with cryptorchidism. Boys were followed up for 2 to 3 years (through January 2008) and orchidopexy was performed in those who were persistently cryptorchid. We analyzed 300 noncryptorchid male children aged 1 to 4 years as controls.

Main Outcome Measures Karyotype anomalies and *INSL3*, INSL3 receptor, and androgen receptor gene mutations.

Results The frequency of genetic alterations in boys with cryptorchidism was low (17/600 [2.8%; 95% confidence interval {CI}, 1.7%-4.5%]) and was significantly higher in participants with persistent cryptorchidism (16/303 [5.3%; 95% CI, 3.0%-8.4%]; $P = .001$) and those with bilateral cryptorchidism (10/120 [8.3%; 95% CI, 4.1%-14.8%]; $P = .001$) than in controls (1/300 [0.3%; 95% CI, 0.1%-0.8%]). Boys with persistent cryptorchidism had a 17-fold greater odds of having a genetic alteration (odds ratio, 16.7; 95% CI, 2.2-126.5). The most common genetic findings in those with cryptorchidism were 8 cases of Klinefelter syndrome and 5 cases of mutations in the INSL3 receptor gene. Genetic alterations were not found in boys with low birth weight or low gestational age, who had frequent spontaneous descent of the testes.

Conclusion In a small percentage of the study population, there was a statistically significant association between bilateral and persistent cryptorchidism and genetic alterations, including Klinefelter syndrome and INSL3 receptor gene mutations.

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RXFP2 and in *AR* genes.² However, published data are not conclusive because most studies involved small cohorts of patients or have been conducted in adult men with a history of cryptorchidism. Furthermore, comprehensive screening for genetic factors associated with cryptorchidism has not been performed.

Therefore, in this study we prospectively screened and followed up a large number of boys with cryptorchidism for karyotype, *INSL3* (RefSeq NM_005543), *RXFP2* (RefSeq NM_130806), and *AR* (RefSeq NM_000044) gene mutations,

relating the findings to the type of cryptorchidism, birth data, and spontaneous descent during the first months after birth.

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METHODS

This study was approved by the local hospital ethics committees and included consecutive boys with cryptorchidism recruited at the departments of pediatric surgery of the University of Messina, Messina, Italy, and University of Padova, Padova, Italy, between January 2003 and March 2005 and followed up until January 2008. Only boys born to Italian parents were recruited to obtain a genetically homogeneous population. Exclusion criteria included multiple births, family history of cryptorchidism, and additional malformations.

Examination of the external genitalia was performed by the same pediatricians (M.R.C. at Messina and G.F.Z. at Padova) and recruitment was standardized between the 2 centers. The boys were examined shortly after birth and at 3 to 6, 12, and 24 months of age. Orchiopexy was performed in boys with persistent cryptorchidism between 2 and 3 years of age. Cryptorchidism was defined as at least 1 testis not being in the scrotum²; therefore, high scrotal testes were considered noncryptorchid.¹ All examinations were done in warm conditions with the child supine.

Information on gestational age and birth weight was collected from birth records, as these factors can influence the risk of cryptorchidism. Gestational age was based on routine ultrasonographic findings during pregnancy, when available, or on the basis of the last menstrual period, and less than 37 weeks was considered preterm. Birth weight less than 2.5 kg was considered low birth weight. Controls were healthy boys aged 1 to 4 years presenting at the same departments of pediatrics for circumcision, voiding problems, or other problems not related to the reproductive system. Each boy had fully descended testes and no urogenital anomalies or known syndromes. Written informed consent was given by the parents of cases and controls.

Blood samples were drawn at birth for cases and at the time of first visit for controls and were analyzed for chromosome alterations by karyotype analy-

sis and for mutations in the *INSL3*, *RXFP2*, and *AR* genes. Genetic analysis was conducted at the Department of Histology, Microbiology, and Medical Biotechnologies of the University of Padova. Karyotype analysis was conducted by standard cytogenetic techniques with the application of GTG and QFQ banding on at least 25 metaphases from peripheral blood lymphocyte culture. Sex chromosome mosaics occurring at a level of less than 5% were not considered. Pericentric inversions of chromosome 9 or other structural chromosomal variants and polymorphisms (such as familial inversion of the Y chromosome or large Y chromosome heterochromatin) were considered normal cytogenetic events. DNA extraction was performed from peripheral blood leukocytes for gene mutation analysis.

For the *INSL3* gene, mutation analysis was performed by polymerase chain reaction (PCR) and direct sequencing of both exons, as previously described.⁶ For the *RXFP2* gene, mutation analysis was focused on exon 8, where the only mutation to date associated with cryptorchidism (T222P) is located. This exon was PCR-amplified and subjected to denaturing high-performance liquid chromatography (DHPLC), as previously described.^{7,8} Abnormal results were confirmed with direct sequencing. For mutation analysis of all 8 exons of the *AR* gene, a combination of methods was used, including PCR and subsequent direct sequencing and DHPLC screening with eventual sequencing, as previously described.^{9,10}

For the prediction of possible impact of amino acid substitutions on the structure and function of the protein, we performed a physical and comparative analysis using the online free software PolyPhen (<http://genetics.bwh.harvard.edu/pph>) that predicts whether the amino acid replacement may be compatible with the spectrum of substitutions observed at the position in the family of homologous proteins. The results of multiple sequence alignment are expressed as the PSIC (position-specific independent counts) score, and

a PSIC score greater than 1.0 indicates a damaging substitution.

Statistical analysis was performed using the statistical software R (<http://cran.r-project.org>). Differences in the frequency of genetic alterations between groups were tested with the Fisher exact test. We performed 48 tests (5 genetic outcomes \times 9 groups plus 3 tests for calculation of odds ratios), which translates into a statistically significant *P* value after Bonferroni correction of $.05/48 \approx .001$. All statistical tests were 2-sided. Odds ratios and the corresponding 95% confidence intervals (CIs) were used to measure the strength of the association.

RESULTS

Among 721 boys with cryptorchidism, 34 parents refused participation. Those with no birth record (15 patients) were excluded, as were 28 twins, 15 boys with a positive family history for cryptorchidism, and 11 with additional congenital malformations. Eighteen boys were lost before first follow-up and therefore excluded from the analysis. The study included 600 singleton boys with isolated nonfamilial cryptorchidism (83.2%) (FIGURE). Three hundred Italian boys without cryptorchidism participated as controls.

Of the 600 boys with cryptorchidism at birth, 396 had unilateral and 204 had bilateral cryptorchidism (Figure). A high percentage of spontaneous descent was observed during the first 3 to 6 months of age (47%), more frequent in unilateral than bilateral cases (199/396 [50.2%] vs 83/204 [40.7%]; *P* = .03). After 6 months of age, 14 (7.1%) of 197 unilateral cryptorchid testes and 1 (0.8%) of 121 bilateral cryptorchid testes spontaneously descended (*P* = .01) (Figure). When spontaneous descent was observed in cases of bilateral cryptorchidism, both testes descended. No spontaneous descent was observed after 12 months of age, so a total of 183 boys with persistently unilateral and 120 boys with persistently bilateral cryptorchidism underwent orchidopexy between 2 and 3 years of age.

No boy was found to carry more than 1 genetic abnormality. The overall frequency of genetic alterations in boys with cryptorchidism was low (17/600 [2.8%; 95% CI, 1.7%-4.5%]) and was statistically significantly higher than controls in participants with persistent cryptorchidism (16/303 [5.3%; 95% CI, 3.0%-8.4%] vs 1/300 [0.3%; 95% CI, 0.1%-0.8%]; $P=.001$) and in those with bilateral cryptorchidism (10/120 [8.3%; 95% CI, 4.1%-14.8%]; $P=.001$) (TABLE 1). As a consequence, the odds ratio of having a genetic alteration in participants with persistent cryptorchidism was 16.7 (95% CI, 2.2-126.5), in participants with unilateral persistent cryptorchidism was 10.1 (95% CI, 1.2-84.9), and in participants with bilateral persistent cryptorchidism was 27.2 (95% CI, 3.4-214.8) (TABLE 2). A post hoc analysis showed that with a prevalence of 0.3% in the 300 controls and 5.3% in the 303 cases with persistent cryptorchidism, the power of the study to detect a difference in the prevalence between cases and controls is 94% with an α level of .05 and 57% with an α level of .001.

We found 8 chromosomal alterations in the cryptorchid group (8/600 [1.3%; 95% CI, 0.6%-2.6%]) and 1 in the control group (1/300 [0.3%; 95% CI, 0.01%-1.8%]). The highest frequency was found in participants with persistent bilateral cryptorchidism (5/120 [4.2%; 95% CI, 1.8%-9.7%]) (Table 1). All chromosomal aberrations found in the cryptorchid group were of the Klinefelter type, either in the complete form (47,XXY; 7 cases) or in the mosaic form (46,XY/47,XXY; 1 case), whereas in controls we found 1 robertsonian translocation involving chromosome 13 and 14 [45,XY,der(13;14)(q10;q10)]. No cases with chromosomal alterations showed spontaneous descent of the testes.

We found 2 mutations in the *INSL3* gene in the cryptorchid group (2/600 [0.3%; 95% CI, 0.04%-1.2%]) and none in the control group (0/300 [0%; 95% CI, 0%-1.2%]) (Table 1). Mutations in the *RXFP2* gene were found in 5 cases (5/600 [0.8%; 95% CI, 0.3%-

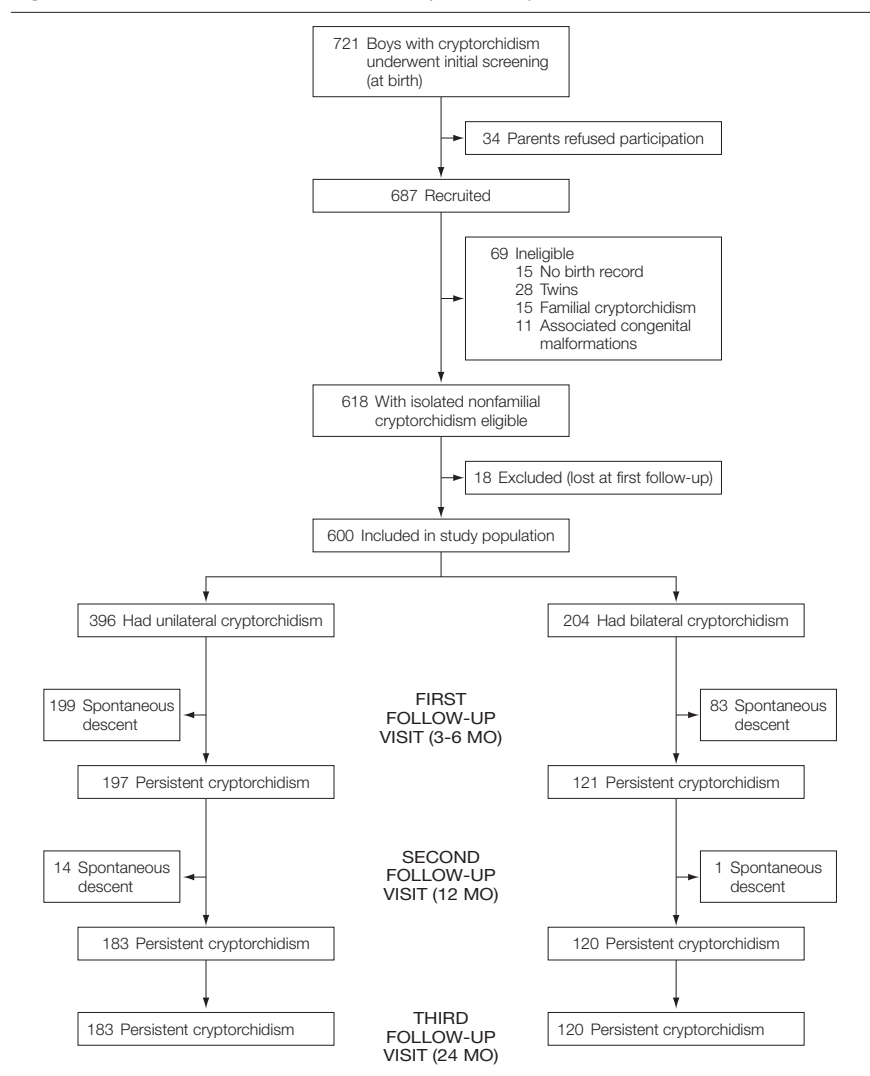
1.9%]) and they were all found in the group of boys with bilateral cryptorchidism (Table 1). The frequency of *RXFP2* mutations in participants with bilateral cryptorchidism at birth was 5 of 204 (2.4%; 95% CI, 0.8%-5.6%) and in boys with bilateral persistent cryptorchidism it was 5 of 120 (4.2%; 95% CI, 1.4%-9.5%). No cases with *RXFP2* mutations showed spontaneous descent of the testes.

Mutations in the *AR* gene were found in 2 cases with unilateral cryptorchidism, whereas no mutations were found in the control group (Table 1). These 2 mutations (H382Y and T558S) rep-

resent novel findings, the first located in the N-terminal domain, the second in the DNA-binding domain of the AR protein. Both of these amino acids are highly conserved, and the observed mutations are predicted to be damaging (PSIC score, 2.354 for H382Y and 1.554 for T558S).

When considering birth weight and gestational age (TABLE 3), boys with low birth weight or low gestational age more frequently underwent spontaneous descent of the testes in the first months of age, and genetic alterations were found exclusively in those with normal weight and gestational age.

Figure. Recruitment, Exclusion, and Follow-up of Participants



Among the 300 controls also participating in this study, there were no dropouts during the study.

Table 1. Prevalence of Genetic Alterations in Cryptorchid Boys at Birth, 3 to 6 Months of Age, and 12 to 24 Months of Age

	Cryptorchidism									Controls (n = 300)
	Unilateral			Bilateral			Total			
	Birth (n = 396)	3-6 mo (n = 197)	12-24 mo (n = 183)	Birth (n = 204)	3-6 mo (n = 121)	12-24 mo (n = 120)	Birth (n = 600)	3-6 mo (n = 318)	12-24 mo (n = 303)	
Karyotype										
No.	3	3	3	5	5	5	8	8	8	1
Prevalence, % (95% CI)	0.8 (0.2-2.2)	1.5 (0.3-4.4)	1.6 (0.3-4.7)	2.5 (0.8-5.6)	4.1 (1.4-9.4)	4.2 (1.4-9.5)	1.3 (0.6-2.6)	2.5 (1.1-4.9)	2.6 (1.2-5.1)	0.3 (0.01-1.8)
P value ^a	.64	.31	.15	.04	.008	.008	.28	.04	.04	
INSL3										
No.	1	1	1	1	0	0	2	1	1	0
Prevalence, % (95% CI)	0.2 (0.01-1.4)	0.5 (0.01-2.8)	0.6 (0.01-3.0)	0.5 (0.01-2.7)	0 (0-3.0)	0 (0-3.0)	0.3 (0.04-1.2)	0.3 (0.01-1.7)	0.3 (0.01-1.8)	0 (0-1.2)
P value ^a	>.99	.40	.38	.40			.55	>.99	>.99	
RXFP2										
No.	0	0	0	5	5	5	5	5	5	0
Prevalence, % (95% CI)	0 (0.0-0.9)	0 (0-1.9)	0 (0-2.0)	2.5 (0.8-5.6)	4.1 (1.4-9.4)	4.2 (1.4-9.5)	0.8 (0.3-1.9)	1.6 (0.5-3.6)	1.6 (0.5-3.8)	0 (0-1.2)
P value ^a				.01	.002	.002	.17	.06	.06	
AR										
No.	2	2	2	0	0	0	2	2	2	0
Prevalence, % (95% CI)	0.5 (0.1-1.8)	1.0 (0.1-3.6)	1.1 (0.1-3.9)	0 (0-1.8)	0 (0-3.0)	0 (0-3.0)	0.3 (0.04-1.2)	0.6 (0.08-2.3)	0.7 (0.08-2.4)	0 (0-1.2)
P value ^a	.51	.16	.14				.55	.49	.49	
Total										
No.	6	6	6	11	10	10	17	16	16	1
Prevalence, % (95% CI)	1.5 (0.6-3.3)	3.0 (1.1-6.5)	3.3 (1.2-7.0)	5.4 (2.7-9.4)	8.3 (4.0-14.7)	8.3 (4.1-14.8)	2.8 (1.7-4.5)	5.0 (0.3-8.0)	5.3 (3.0-8.4)	0.3 (0.1-0.8)
P value ^a	.25	.02	.01	.001	.001	.001	.01	.001	.001	

Abbreviations: AR, androgen receptor gene, CI, confidence interval; INSL3, insulin-like factor 3 gene, RXFP2, relaxin family peptide 2 gene.
^aP values are for comparison of cases and controls.

Table 2. Odds of Genetic Alteration in Persistently Cryptorchid Boys (12-24 Months of Age)

Cryptorchidism	No. (%)	Odds Ratio (95% Confidence Interval)
Unilateral (n = 183)	6 (3.3)	10.1 (1.2-84.9)
Bilateral (n = 120)	10 (8.3)	27.2 (3.4-214.8)
Total (n = 303)	16 (5.3)	16.7 (2.2-126.5)
Controls (n = 300)	1 (0.3)	1 [Reference]

COMMENT

In this study, we found genetic alterations in a small percentage of boys with cryptorchidism. We found a significant association between bilateral and persistent cryptorchidism and genetic alterations, including mutations in the INSL3 receptor gene (*RXFP2*) and Klinefelter syndrome. Genetic alterations were not found in participants with low birth weight or low gestational age, who had frequent spontaneous descent of the testes.

Mutations in the *INSL3* gene were found in only 2 cases in this study. One

mutation (R102C), detected in a boy with unilateral persistent cryptorchidism, has been previously described as associated with cryptorchidism,^{6,11} whereas the other mutation (T86M), detected in a boy with bilateral cryptorchidism at birth and spontaneous descent in the first months of age, is a novel finding. This residue is located in the C-peptide of the protein and it is poorly conserved: only humans have a T in this position, whereas other species harbor A, V, L, or even M.¹² These data, together with the mild phenotype of the participant with this

substitution, suggest considering with caution this mutation as pathogenetic for cryptorchidism. Insulin-like factor 3 is produced, like testosterone, by the Leydig cells of the testis and regulates the transabdominal phase of testicular descent during fetal life. Mice without the gene encoding this hormone or its receptor exhibit bilateral cryptorchidism with abdominally located testes.¹³⁻¹⁵ A recent review of the literature showed that mutations in the *INSL3* gene are found in 1.8% (30/1650) of cases of cryptorchidism,² a number substantially higher than that found in the present study. Although the reason for this discrepancy is not obvious, a direct comparison of the data is not possible because a large number of cases reported in the literature were adult men with history of cryptorchidism often recruited in infertility clinics. Nevertheless, the role of *INSL3* in testicular descent is well estab-

Table 3. Prevalence of Genetic Alterations in Cryptorchid Boys by Follow-up Period on the Basis of Birth Weight and Gestational Age

	Genetic Alterations, No./Total (%)								
	Unilateral			Bilateral			Total		
	Birth	3-6 mo	12-24 mo	Birth	3-6 mo	12-24 mo	Birth	3-6 mo	12-24 mo
Birth weight <2.5 kg, gestational age <37 wk	0/35	0/0	0/0	0/41	0/2	0/2	0/76	0/2	0/2
Birth weight <2.5 kg, gestational age ≥37 wk	0/7	0/0	0/0	0/5	0/1	0/1	0/12	0/1	0/1
Birth weight ≥2.5 kg, gestational age <37 wk	0/9	0/1	0/1	0/7	0/3	0/3	0/16	0/4	0/4
Birth weight ≥2.5 kg, gestational age ≥37 wk	6/345 (1.7)	6/196 (3.1)	6/182 (3.3)	11/151 (7.3)	10/115 (8.7)	10/114 (8.8)	17/496 (3.4)	16/311 (5.1)	16/296 (5.4)
Total	6/396 (1.5)	6/197 (3.1)	6/183 (3.3)	11/204 (5.4)	10/121 (8.3)	10/120 (8.3)	17/600 (2.8)	16/318 (5.0)	16/303 (5.3)

lished and disruption of this hormonal pathway might be involved in the pathogenesis of cryptorchidism through mechanisms other than gene mutations. For example, INSL3 concentrations are reduced in cord blood of boys with persistent cryptorchidism.¹⁶ It has been suggested that environmental factors acting as endocrine disruptors might cause difficulties in testicular descent by suppressing INSL3 production by the Leydig cells.¹⁷

The fundamental role of INSL3 in regulating testicular descent is also highlighted by the finding in this study of mutations in the gene for its receptor, *RXFP2*, especially in the bilateral forms of cryptorchidism. Mutation screening for this gene in humans has shown a unique missense mutation (T222P) associated with cryptorchidism, with a prevalence of about 3% in boys with cryptorchidism or adult men with history of cryptorchidism.² In the present study, we found a prevalence of 4% for the T222P mutation in the *RXFP2* gene in participants with persistent bilateral cryptorchidism. In addition to cryptorchidism, individuals with *RXFP2* mutations frequently have reduced bone mass early in adulthood.¹⁸

Although the role of androgens in male phenotype development and testicular descent is well known,² only 2 small studies involving a total of 69 cases looked at mutations in the *AR* gene in boys with isolated cryptorchidism,^{19,20} and they failed to find any abnormality. Mutations in the *AR* gene

cause varying degrees of androgen insensitivity, from the more severe forms with complete female phenotype to partial forms with micropenis, hypospadias, and cryptorchidism, and to mildest forms with only spermatogenic impairment. In cases of *AR* mutations, cryptorchidism therefore might represent 1 sign of a complex syndrome or it may not be found even in more severe forms of androgen insensitivity.²¹ In one study, 1.63% (2/123) of infertile adult men with a history of cryptorchidism had *AR* mutations, a frequency not different from that of infertile men without a history of cryptorchidism (24/1437 [1.67%]).⁹ These data, together with the findings of the present study, suggest that *AR* mutations are not a frequent cause of isolated cryptorchidism.

We found that chromosomal aberrations represent the most frequent genetic alteration in participants with isolated cryptorchidism, particularly in those with persistent cryptorchidism (1.6% in the unilateral forms and 4.2% in the bilateral forms) and that chromosomal alterations were exclusively represented by Klinefelter syndrome. The prevalence of Klinefelter syndrome in the general population is 1 in 500,^{22,23} but the prevalence of cryptorchidism at birth in patients with Klinefelter syndrome is unclear, as few studies have examined this aspect. The largest cross-sectional study undertaken in an andrological clinic reported that 27% of patients with Klinefelter syndrome had a history of

undescended testes compared with 8% of the total number of patients who attended the same clinic.²² Klinefelter syndrome is an underdiagnosed condition; only 25% of the expected number of cases are diagnosed,²⁴ and of these only a minority are diagnosed before puberty. Nevertheless, diagnosis of Klinefelter syndrome early in childhood would allow better management of these patients during puberty, preservation of fertility, and initiation of androgen replacement therapy before symptoms and signs of hypogonadism develop.

Our findings have the limitations of a case-control study, and the main limitation is the small number of genetic abnormalities found. Future studies involving a higher number of participants are necessary to confirm our findings. This study should therefore be considered preliminary, and strong conclusions about association cannot be drawn.

Although we are far from understanding the etiology of a large proportion of cases of cryptorchidism, we propose a distinction between intrinsic and extrinsic causes. The first group, which includes genetic alterations, frequently displays bilateral cryptorchidism and is associated with progressive testicular damage (also of the normally descended testis in cases of unilateral cryptorchidism),²⁵ with increased risk of infertility and testicular cancer.²⁶⁻³⁰ In these cases, early orchidopexy might reduce the risk of these sequelae but does not abolish it.

Genetic alterations causing this type of cryptorchidism might be associated with other pathologies in adulthood, as in the case of Klinefelter syndrome and *RXFP2* gene mutations. The second group, which includes low birth weight, prematurity, or complications during pregnancy such as maternal diabetes or preeclampsia, frequently has spontaneous descent in the first months of age and has a minor risk of testicular degeneration, especially if orchidopexy is performed early.³⁰ Genetic causes in these cases are far less frequent.

Author Contributions: Dr Ferlin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Ferlin, B. Zuccarello, Foresta. **Acquisition of data:** Ferlin, D. Zuccarello, B. Zuccarello, Chirico.

Analysis and interpretation of data: Ferlin, D. Zuccarello, Zanon, Foresta.

Drafting of the manuscript: Ferlin, D. Zuccarello, Foresta.

Critical revision of the manuscript for important intellectual content: B. Zuccarello, Chirico, Zanon, Foresta.

Statistical analysis: Ferlin.

Obtained funding: Foresta.

Administrative, technical, or material support: D. Zuccarello, B. Zuccarello, Chirico.

Study supervision: Ferlin, B. Zuccarello, Zanon, Foresta.

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REFERENCES

- Boisen KA, Kaleva M, Main KM, et al. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet*. 2004;363(9417):1264-1269.
- Foresta C, Zuccarello D, Garolla A, Ferlin A. Role of hormones, genes and environment in human cryptorchidism. *Endocr Rev*. 2008;29(5):560-580.
- Virtanen HE, Toppari J. Epidemiology and pathogenesis of cryptorchidism. *Hum Reprod Update*. 2008;14(1):49-58.
- Klonisch T, Fowler PA, Hombach-Klonisch S. Molecular and genetic regulation of testis descent and external genitalia development. *Dev Biol*. 2004;270(1):1-18.
- Cortes D, Kjellberg EM, Breddam M, Thorup J. The true incidence of cryptorchidism in Denmark. *J Urol*. 2008;179(1):314-318.
- Ferlin A, Bogatcheva NV, Giansello L, et al. Insulin-like factor 3 gene mutations in testicular dysgenesis syndrome: clinical and functional characterization. *Mol Hum Reprod*. 2006;12(6):401-406.
- Ferlin A, Simonato M, Bartoloni L, et al. The *INSL3-LGR8/GREAT* ligand-receptor pair in human cryptorchidism. *J Clin Endocrinol Metab*. 2003;88(9):4273-4279.
- Bogatcheva NV, Ferlin A, Feng S, et al. T222P mutation of the insulin-like 3 hormone receptor *LGR8* is associated with testicular maldescent and hinders receptor expression on the cell surface membrane. *Am J Physiol Endocrinol Metab*. 2007;292(1):E138-E144.
- Ferlin A, Vianzi C, Garolla A, et al. Male infertility and androgen receptor gene mutations: clinical features and identification of seven novel mutations. *Clin Endocrinol (Oxf)*. 2006;65(5):606-610.
- Ferlin A, Garolla A, Bettella A, et al. Androgen receptor gene *CAG* and *GGC* repeat lengths in cryptorchidism. *Eur J Endocrinol*. 2005;152(3):419-425.
- Bogatcheva NV, Truong A, Feng S, Engel W, Adham IM, Agoulnik AI. *GREAT/LGR8* is the only receptor for insulin-like 3 peptide. *Mol Endocrinol*. 2003;17(12):2639-2646.
- Truong A, Bogatcheva NV, Schelling C, Dolf G, Agoulnik AI. Isolation and expression analysis of the canine insulin-like factor 3 gene. *Biol Reprod*. 2003;69(5):1658-1664.
- Nef S, Parada LF. Cryptorchidism in mice mutant for *INSL3*. *Nat Genet*. 1999;22(3):295-299.
- Zimmermann S, Steding G, Emmen JM, et al. Targeted disruption of the *INSL3* gene causes bilateral cryptorchidism. *Mol Endocrinol*. 1999;13(5):681-691.
- Overbeek PA, Gorlov IP, Sutherland RW, et al. A transgenic insertion causing cryptorchidism in mice. *Genesis*. 2001;30(1):26-35.
- Bay K, Virtanen HE, Hartung S, et al; Nordic Cryptorchidism Study Group. Insulin-like factor 3 levels in cord blood and serum from children: effects of age, postnatal hypothalamic-pituitary-gonadal axis activation, and cryptorchidism. *J Clin Endocrinol Metab*. 2007;92(10):4020-4027.
- Sharpe RM. The "oestrogen hypothesis"—where do we stand now? *Int J Androl*. 2003;26(1):2-15.
- Ferlin A, Pepe A, Giansello L, et al. Mutations in the insulin-like factor 3 receptor are associated with osteoporosis. *J Bone Miner Res*. 2008;23(5):683-693.
- Wiener JS, Marcelli M, Gonzales ET Jr, Roth DR, Lamb DJ. Androgen receptor gene alterations are not associated with isolated cryptorchidism. *J Urol*. 1998;160(3 pt 1):863-865.
- Suzuki Y, Sasagawa I, Ashida J, Nakada T, Muroya K, Ogata T. Screening for mutations of the androgen receptor gene in patients with isolated cryptorchidism. *Fertil Steril*. 2001;76(4):834-836.
- Barthold JS, Kumasi-Rivers K, Upadhyay J, Shekarriz B, Imperato-Mcginley J. Testicular position in the androgen insensitivity syndrome: implications for the role of androgens in testicular descent. *J Urol*. 2000;164(2):497-501.
- Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. *Lancet*. 2004;364(9430):273-283.
- Nielsen J, Wohlert M. Sex chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. In: Evans JA, Hamerton JL, eds. *Children and Young Adults With Sex Chromosome Aneuploidy*. New York, NY: Wiley-Liss for the March of Dimes Birth Defects Foundation; 1991:209-223. Birth Defects original article series volume 26.
- Bojesen A, Gravholt CH. Klinefelter syndrome in clinical practice. *Nat Clin Pract Urol*. 2007;4(4):192-204.
- Foresta C, Ferlin A, Garolla A, Milani C, Oliva G, Rossato M. Functional and cytologic features of the contralateral testis in cryptorchidism. *Fertil Steril*. 1996;66(4):624-629.
- Giwerzman A, von der Maase H, Skakkebaek NE. Epidemiological and clinical aspects of carcinoma in situ of the testis. *Eur Urol*. 1993;23(1):104-110.
- Cortes D, Thorup JM, Visfeldt J. Cryptorchidism: aspects of fertility and neoplasms: a study including data of 1,335 consecutive boys who underwent testicular biopsy simultaneously with surgery for cryptorchidism. *Horm Res*. 2001;55(1):21-27.
- Rajpert-De Meyts E. Developmental model for the pathogenesis of testicular carcinoma in situ: genetic and environmental aspects. *Hum Reprod Update*. 2006;12(3):303-323.
- Husmann DA. Cryptorchidism and its relationship to testicular neoplasia and microlithiasis. *Urology*. 2005;66(2):424-426.
- Walsh TJ, Dall'Era MA, Croughan MS, Carroll PR, Turek PJ. Prepubertal orchidopexy for cryptorchidism may be associated with lower risk of testicular cancer. *J Urol*. 2007;178(4 pt 1):1440-1446.