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The Leucine-Rich Repeat-Containing G Protein-Coupled Receptor 8 Gene T222P Mutation Does Not Cause Cryptorchidism

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Context: Insulin-like 3 and its receptor, leucine-rich repeat-containing G protein-coupled receptor 8 (LGR8), are essential for the first phase of testicular descent. Homozygous loss of either of the two genes in mice leads to cryptorchidism. Although mutations in both homologous human genes are not a common cause of cryptorchidism. To date, only one missense mutation at codon 222 (T222P) of the LGR8 gene has been proposed as a causative mutation for cryptorchidism. This conclusion was based on both functional *in vitro* studies and the lack of mutation in a large group of controls. The geographical origin of the mutation carriers suggested a founder effect in the Mediterranean area.

Objectives: We sought to define the frequency of the T222P mutation in four different countries to assess whether the screening for this mutation could be of use as a diagnostic genetic test.

Materials and Methods: A total of 822 subjects (359 with a history of cryptorchidism and 463 controls) from Italy, Spain, Hungary, and Egypt were genotyped for the T222P mutation by direct sequencing.

Results: The phenotypical expression of the mutation also included normal testicular descent. The mutation frequency was not significantly different in cryptorchid patients vs. noncryptorchid controls (3.6 vs. 1.7%, respectively). No significant geographical differences were observed in mutation frequencies. The haplotype analysis allowed us to predict three distinct haplotypes, *i.e.* three possible mutation events.

Conclusions: Our results suggest that the T222P mutation cannot be considered either causative or a susceptibility factor for cryptorchidism. A true causative mutation in the LGR8 gene still remains to be identified. (*J Clin Endocrinol Metab* 93: 1072–1076, 2008)

Cryptorchidism is the most common congenital malformation in newborn boys (1–9%) and may occur as an isolated anomaly or may be associated with other congenital disorders (1). The etiology of the majority of nonsyndromic (isolated) cryptorchidism remains unknown, but many risk factors, both environmental and genetic, have been proposed (1–3).

Testicular descent requires the action of two major hormonal factors: insulin-like 3 (INSL3) and androgens. INSL3 and its receptor, the leucine-rich repeat-containing G protein-coupled receptor 8 (LGR8), recently renamed relaxin family peptide receptor 2 (4), are essential for the first phase (transabdominal) of descent, whereas androgens (mainly testosterone) and the an-

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Abbreviations: INSL3, Insulin-like 3; LGR8, leucine-rich repeat-containing G protein-coupled receptor 8.

drogen receptor act principally during the second (inguinoscrotal) phase (5). Any factor or genetic anomaly influencing these two hormone receptor systems may lead to cryptorchidism. Certain androgen receptor gene mutations in human are associated with cryptorchidism, but in these cases, other symptoms of androgen deficiency are also present (6). The first evidence supporting the importance of the INSL3/LGR8 system derives from animal models. Knockout mutant mice for either *Insl3* (7, 8) or *Lgr8* (9, 10) are cryptorchid, and the overexpression of *Insl3* in the female mouse induces ovarian descent (11, 12). Moreover, *Lgr8* is highly expressed in the gubernaculum, the ligament that controls testicular position during development, and the gubernacula of the mutant males fail to differentiate. Despite this, mutations in both homologous human genes are not a common cause of cryptorchidism in patients. Since the first mutation screening of the *INSL3* gene in cryptorchid men (13), a large number of polymorphisms and putative mutations were described (5, 14). Of all reported mutations, only two missense, the P49S and V18M, mutations had a deleterious effect on the ability of INSL3 to activate its receptor (15, 16). However, the role of these mutations (all heterozygous) in the pathogenesis of cryptorchidism remains to be established. The screening for *LGR8* mutations was also relatively disappointing because apart from a few genetic variants with no clear pathogenic effect, only one putative causative missense mutation at codon 222 (T222P) was described in subjects from Southern Europe (9, 17). All patients were heterozygous for the mutation and presented a variable phenotype of testicular maldescent. Because the haplotype analysis indicated a common origin of this mutation, a founder effect in the Mediterranean area was postulated. Functional studies showed a severely reduced receptor surface expression of the mutant protein supporting its causative role in the pathogenesis of cryptorchidism (18). The functional data, together with the lack of the T222P mutation in 450 controls from Italy, led to the conclusion of an exclusive association of this mutation with cryptorchidism. Based on the aforementioned conclusion, Bogatcheva *et al.* (18) suggested that the recognition of such patients would permit family screening and potential early therapeutic intervention.

The aim of the present study was to evaluate the frequency of the T222P mutation in four different countries (Italy, Spain, Hungary, and Egypt), both for clinical purposes and for gaining further insight into the origin of this “Mediterranean” mutation.

Patients and Methods

A total of 822 subjects from four different countries were analyzed for the T222P mutation. Among them 359 patients had a history of cryptorchidism, whereas the remaining 463 were controls with no history of testicular maldescent at birth. The geographical distribution was: 1) 159 patients of Italian origin ($n = 98$ unilateral, $n = 61$ bilateral) attending the Andrology Unit of the University Hospital Careggi (Florence) and from the Endocrinology Unit of the University of Ancona; 2) 70 Hungarian patients ($n = 51$ unilateral, $n = 19$ bilateral) from the Andrology Division of the National Health Center of Budapest; 3) 53 patients of Egyptian origin ($n = 45$ unilateral, $n = 8$ bilateral) from the Kamal Hospital; and 4) 77 Spanish patients from the Fundacio Puigvert (Barcelona). The controls, without a history of cryptorchidism at birth, *i.e.*

also excluding late spontaneous descent or retractile testis, were: 1) group A, 312 patients (144 Italian, 120 Hungarian, and 48 Spanish) with reduced sperm count from infertile couples; and 2) group B, 151 (131 Italian, 20 Hungarian) with normal sperm parameters. Written informed consent was obtained from all patients and controls included in the present study. The institutional review boards of referral centers have approved this protocol.

Genotyping

The sequences of the primers used for the amplification of exon 8 containing the T222P mutation were: forward, 5'-GGGGAGGCAG-GTTTTATTTC-3'; and reverse, 5'-AAGCTAGTGCTAGATGTCAT-TGC-3'. The resulting DNA fragments were sequenced using automated sequencer (ABI PRISM 310; Applied Biosystems, Foster City, CA).

Haplotype analysis

A total of five polymorphisms were analyzed in 19 carriers of the T222P mutation. For the analysis of exon 12-intron 12 polymorphisms [exon 12: 957G>A, 993A>G; intron 12: INV12(-2)A₉>A₁₃], the primers are described by Ferlin *et al.* (17). Primers for the intron 7 [INV7(-351)A>C] and intron 9 polymorphisms [INV9(+380)A>C] were as follows: forward, 5'-ATCAGTTTAACACCATGTGACCAAA-3'; reverse, 5'-GGGTACCTGGGTCTGGCAC-3'; and forward, 5'-CCCTAAGCATATTGTTCTTGA-3'; reverse, 5'-GGGAGTACAT-AGGTGGCTGCTG-3'. The resulting DNA fragments were sequenced using automated sequencer (ABI PRISM 310).

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, IL). Genotype and allele frequencies were analyzed by the χ^2 test. Between-group comparisons for semen parameters were performed by the Mann-Whitney *U* test for unpaired data. A *P* value less than 0.05 was considered statistically significant.

Results

Mutation analysis of exon 8 of the *LGR8* gene

The *LGR8* mutation analysis was performed in 822 subjects from three Mediterranean and one Central European country. The composition of the study population is reported in Table 1. The T222P mutation was found in both cryptorchid and non-cryptorchid subjects. The frequency observed in the cryptorchid group ($n = 359$) was 3.6%, which is not significantly different from the 1.7% in 463 controls (groups A and B). The mutation frequencies in different subgroups of controls and patients based on their ethnic background are reported in Table 1. The highest mutation frequency among cryptorchids was found in the Egyptian population (5.66%), followed by the Italian (5.03%) and Hungarian (2.86%). No mutation was found in the Spanish cryptorchids. In the control groups, we found the highest frequency in the Spanish population (4.17%) (likely due to the small sample size), followed by Italians (1.82%) and Hungarians (0.71%). Although Hungarians showed a relatively low frequency, the difference was not significant in comparison with other countries. Finally, to define whether the T222P mutation can be considered a risk factor in a specific ethnic group, we compared the mutation frequencies between cryptorchids and noncryptorchids for each country. The observed differences were not statistically significant in any of the examined populations.

TABLE 1. T222P LGR8 mutation in patients with a history of testicular maldescent and in controls with no history of cryptorchidism, or retractile testes or late spontaneous descent

	No. of mutated/ no. of total men	T222P mutation frequency (%)
Cryptorchid (n = 359)		
Italians	8/159	5.03
Hungarians	2/70	2.86
Spanish	0/77	0
Egyptians	3/53	5.66
Total	13/359	3.6
Noncryptorchid (n = 463)		
Italians (n = 275)		
Group A	2/131	1.52
Group B	3/144	2.08
Hungarians (n = 140)		
Group A	0/20	0
Group B	1/120	0.83
Spanish (n = 48)		
Group B	2/48	4.17
Total	8/463	1.7

The "non cryptorchid" control group includes group A, which contains patients with reduced sperm count referred for couple infertility, and group B, which contains men with normal sperm parameters.

Genotype/phenotype correlation

In all 21 cases, the mutation was heterozygous. Analysis of the parent's DNA was possible only in one patient, and the mother resulted a heterozygous carrier. The brother of patient A251 had a history of bilateral cryptorchidism as well. The phenotypical expression of this mutation in our multiethnic study population also includes normal testicular descent in eight cases from three different countries. These data exclude a causative association between the mutation and cryptorchidism. The testicular phenotype of the 13 cryptorchid patients was variable: seven had a history of unilateral and six of bilateral cryptorchidism. In all cases orchidopexy was performed during infancy. The sperm concentration of the cryptorchid mutation carriers ranged from azoospermia to 11 million spermatozoa per milliliter, whereas in the noncryptorchids from 0.9–75 million spermatozoa per milliliter. No significant difference was found in the mean sperm concentration and total sperm count between cryptorchid men with and without mutation. The frequency of the T222P mutation in the noncryptorchid controls was similar, regardless of the level of sperm production: 1.9% in patients with impaired sperm production (group A) vs. 1.3% in normozoospermic men (group B).

LGR8 gene haplotyping

The analysis of five exonic and intronic polymorphisms in 19 carriers allowed the discrimination of different possible haplotypes (Table 2). The majority of patients and controls (n = 16) presented a common inferred haplotype: C-C-G-A-13. Two Hungarian cryptorchid men (HU85, HU89) were lacking this specific haplotype due to a different length of the polyadenine stretch. These two men share the C-C-G-A-10 haplotype that may also derive from the common C-C-G-A-13 through the contraction of the polyadenine stretch. Finally, one control patient of Italian origin (A448) resulted homozygous for the INV9 (+380) marker (AA), consequently, his inferred haplotypes are

as follows: C-A-G-A-13 and C-A-G-A-10. The C-A-G-A-13 haplotype is also shared by seven other patients. According to our results, the mutation must have occurred on at least two (or three) independent occasions.

Discussion

Since the first description of the T222P mutation of the *LGR8* gene, a clear-cut cause-effect relationship was evoked between the mutation and cryptorchidism (9, 17, 18). This conclusion was based on the lack of this mutation in a relatively large group (n = 643) of unaffected controls from Italy, the United States, and France. Given that all mutations in humans were heterozygous, whereas cryptorchidism was observed exclusively in homozygous knockout *Lgr8* mice, a number of hypotheses were proposed to explain this contrast (17). The most likely among them seemed to be haploinsufficiency (threshold effect), *i.e.* the absence of one allele is enough to cause the phenotype. Recent *in vitro* functional studies demonstrated that the mutation, rather than influencing receptor signal transduction, reduces receptor expression on cell membranes (18). This would support the threshold hypothesis, and also explain the wide variability ranging from bilateral cryptorchidism to unilateral maldescent and to retractile testes. According to the first studies, the phenotypical expression of the mutation was restricted to cryptorchidism, also in the last largest study in which no mutation was found in 450 Italian controls (18). This figure is in sharp contrast with our finding of a total of eight carriers of 463 noncryptorchid men (eight of 463 vs. zero of 450; $P < 0.01$) and remains highly significant, even if we restrict the comparison to the Italian controls (five of 275 vs. zero of 450; $P < 0.01$). The relatively high frequency of the mutation (>1%, with the exception of Hungary) in men without a history of cryptorchidism or retractile testes indicates that the T222P mutation is more likely to be a polymorphism in these countries rather than a causative mutation. Because a mild functional effect was reported in *in vitro* studies, it is still possible that only homozygous T222P mutation (not yet found) is associated with abnormal testis descent. It is intriguing that the mutation is also absent in a total of 139 cryptorchid and 286 control subjects from Germany, France, Finland, the United States, and Japan (9, 19–21). This would support the initial hypothesis of a founder effect in the Mediterranean area. However, the presence of the mutation in a central European population such as Hungary, together with the haplotype analysis, questions the existence of a unique common ancestor. Our analysis indicates that the mutation occurred at least twice (more likely three times) during human history. However, an alternative explanation could be that recurrent mutation at the INV9 (+380) position converting CCGA13 to CAGA13 or gene conversion producing local changes to the haplotypes may have occurred, and, thus, the mutations have originated in one single individual.

The original aim of our study was to define the frequency of the T222P mutation of the *LGR8* gene, the only known "causative" mutation for nonsyndromic cryptorchidism, in four different countries, to assess whether the screening for this mutation could be of use as a diagnostic genetic test. Although the frequency we found in

TABLE 2. Haplotype analysis of the LGR8 gene based on five intronic (intron 7 and 9) and exonic (exon 12) polymorphisms

Code	Polymorphisms				Possible haplotypes
	INV7 (–351) A>C	INV9 (+380) A>C	Exon 12 SNPs 957G>A 993A>G	Intron 12 INV12 (–2)A ₉ >A ₁₃	
Italian					
Cryptorchid					
MMP 122	AC	AC	GG AA	12/13	AAGA12/ACGA12/CAGA12/CCGA12 AAGA13/ACGA13/CAGA13/ CCGA13
MMP 299	AC	CC	GG AA	10/13	ACGA10/CCGA10/ACGA13/ CCGA13
MMP 475	AC	AC	GG AA	12/13	AAGA12/ACGA12/CAGA12/CCGA12 AAGA13/ACGA13/CAGA13/ CCGA13
A191	AC	CC	GG AA	10/13	ACGA10/CCGA10/ACGA13/ CCGA13
A 251	CC	CC	GA AA	12/13	CCGA12/CCAA12/ CCGA13 /CCAA13
A639	AC	CC	GG AA	10/13	ACGA10/CCGA10/ACGA13/ CCGA13
CH 4	AC	CC	GA AA	10/13	ACGA10/CCGA10/ACAA10/CCAA10 ACGA13/ CCGA13 /ACAA13/CCAA13
CH 16	AC	AC	GG AA	12/13	AAGA12/ACGA12/CAGA12/CCGA12 AAGA13/ACGA13/CAGA13/ CCGA13
Control 1					
A268	AC	CC	GG AA	11/13	ACGA11/CCGA11/ACGA13/ CCGA13
A337	AC	CC	GG AA	10/13	ACGA10/CCGA10/ACGA13/ CCGA13
A448	CC	AA	GG AA	10/13	CAGA10/CAGA13
Control 2					
CS32	CC	AC	GG GA	11/13	CAGG11/CCGG11/CAGA11/CCGA11 CAGG13/CCGG13/CAGA13/ CCGA13
C68	AC	AC	GA AA	10/13	AAGA10/CAGA10/ACGA10/CCGA10 AAAA10/CAAA10/ACAA10/CCAA10 AAGA13/CAGA13/ACGA13/ CCGA13 AAAA13/CAAA13/ACAA13/CCAA13
Hungarian					
Cryptorchid					
HU85	AC	AC	GA AA	10/12	AAGA10/ACGA10/CAGA10/ CCGA10 AAAA10/ACAA10/CAAA10/CCAA10 AAGA12/ACGA12/CAGA12/CCGA12 AAAA12/ACAA12/CAAA12/CCAA12
HU89	AC	AC	GA AA	10/10	AAGA10/ACGA10/CAGA10/ CCGA10 AAAA10/ACAA10/CAAA10/CCAA10
Control 1					
HU179	AC	CC	GG GA	11/13	ACGG11/CCGG11/ACGA11/CCGA11 ACGG13/CCGG13/ACGA13/ CCGA13
Egyptian					
Cryptorchid					
E630	AC	CC	GG AA	12/13	ACGA12/CCGA12/ACGA13/ CCGA13
E371	AC	AC	GG AA	12/13	AAGA12/ACGA12/CAGA12/CCGA12 AAGA13/ACGA13/CAGA13/ CCGA13
Spanish					
Control 1					
SP169	CC	AC	GG GA	11/13	CCGG11/CAGG11/CCGA11/CAGA11 CCGG13/CAGG13/ CCGA13 /CAGA13

Control 1 represents noncryptorchid patients with impaired sperm production. Control 2 represents normospermic noncryptorchid. Common inferred haplotypes are indicated in *bold*. SNP, Single nucleotide polymorphism.

the cryptorchid groups is similar to that of Ferlin *et al.* (17), our results also suggest that the T222P mutation cannot be considered either causative or a susceptibility factor for cryptorchidism. Despite the well-established role of the INSL3/LGR8 system in the regulation of testicular descent, a true causative mutation in the *LGR8* gene still remains to be identified.

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References

1. Kaleva M, Toppari J 2003 Genetics and hormones in testicular descent. *Hormones (Athens)* 2:211–216
2. Ivell R, Hartung S 2003 The molecular basis of cryptorchidism. *Mol Hum Reprod* 9:175–181
3. Bogatcheva NV, AgoulNIK AI 2005 INSL3/LGR8 role in testicular descent and cryptorchidism. *Reprod Biomed Online* 10:49–54
4. Bathgate RA, Ivell R, Sanborn BM, Sherwood OD, Summers RJ 2006 International Union of Pharmacology LVII: recommendations for the nomenclature of receptors for relaxin family peptides. *Pharmacol Rev* 58:7–31
5. Adham IM, AgoulNIK AI 2004 Insulin-like 3 signalling in testicular descent. *Int J Androl* 27:257–265
6. Gottlieb B, Beitel LK, Wu JH, Trifiro M 2004 The androgen receptor gene mutations database (ARDB): 2004 update. *Hum Mutat [Erratum (2004) 24:102]* 23:527–533
7. Nef S, Parada LF 1999 Cryptorchidism in mice mutant for Insl3. *Nat Genet* 22:295–299
8. Zimmermann S, Steding G, Emmen JM, Brinkmann AO, Nayernia K, Holstein AF, Engel W, Adham IM 1999 Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. *Mol Endocrinol* 13:681–691
9. Gorlov IP, Kamat A, Bogatcheva NV, Jones E, Lamb DJ, Truong A, Bishop CE, McElreavey K, AgoulNIK AI 2002 Mutations of the GREAT gene cause cryptorchidism. *Hum Mol Genet* 11:2309–2318
10. Hsu SY, Nakabayashi K, Nishi S, Kumagai J, Kudo M, Sherwood OD, Hsueh AJ 2002 Activation of orphan receptors by the hormone relaxin. *Science* 295:671–674
11. Adham IM, Steding G, Thamm T, Bullesbach EE, Schwabe C, Paprotta I, Engel W 2002 The overexpression of the insl3 in female mice causes descent of the ovaries. *Mol Endocrinol* 16:244–2452
12. Ivell R, Hartung S, Anand-Ivell R 2005 Insulin-like factor 3: where are we now? *Ann NY Acad Sci* 1041:486–496
13. Krausz C, Quintana-Murci L, Fellous M, Siffroi JP, McElreavey K 2000 Absence of mutations involving the INSL3 gene in human idiopathic cryptorchidism. *Mol Hum Reprod* 6:298–302
14. Ferlin A, Arredi B, Zuccarello D, Garolla A, Selice R, Foresta C 2006 Paracrine and endocrine roles of insulin-like factor 3. *J Endocrinol Invest* 29:657–664
15. El Houate B, Rouba H, Sibai H, Barakat A, Chafik A, Chadli el B, Imken L, Bogatcheva NV, Feng S, AgoulNIK AI, McElreavey K 2007 Novel mutations involving the INSL3 gene associated with cryptorchidism. *J Urol* 177:1947–1951
16. Bogatcheva NV, Truong A, Feng S, Engel W, Adham IM, AgoulNIK AI 2003 GREAT/LGR8 is the only receptor for insulin-like 3 peptide. *Mol Endocrinol* 17:2639–2646
17. Ferlin A, Simonato M, Bartoloni L, Rizzo G, Bettella A, Dottorini T, Dallapiccola B, Foresta C 2003 The INSL3-LGR8/GREAT ligand-receptor pair in human cryptorchidism. *J Clin Endocrinol Metab* 88:4273–4279
18. Bogatcheva NV, Ferlin A, Feng S, Truong A, Ganesello L, Foresta C, AgoulNIK AI 2007 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* 292:E138–E144
19. Roh J, Virtanen H, Kumagai J, Sudo S, Kaleva M, Toppari J, Hsueh AJ 2003 Lack of LGR8 gene mutation in Finnish patients with a family history of cryptorchidism. *Reprod Biomed Online* 7:400–406
20. Feng S, Cortessis VK, Hwang A, Hardy B, Koh CJ, Bogatcheva NV, AgoulNIK AI 2004 Mutation analysis of INSL3 and GREAT/LGR8 genes in familial cryptorchidism. *Urology* 64:1032–1036
21. Yamazawa K, Wada Y, Sasagawa I, Aoki K, Ueoka K, Ogata T 2007 Mutation and polymorphism analyses of INSL3 and LGR8/GREAT in 62 Japanese patients with cryptorchidism. *Horm Res* 67:73–76