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1 Isolated Cryptorchidism: No Evidence for Involvement of Genes

2 Underlying Idiopathic Hypogonadotropic Hypogonadism

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Abstract

Mutations in *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, or *TACR3* underlie isolated hypogonadotropic hypogonadism (IHH) with clinically variable phenotypes, and, by causing incomplete intrauterine activation of the hypothalamic – pituitary – testicular axis, may lead to cryptorchidism. To investigate the role of defects in these genes in the etiology of isolated cryptorchidism, we screened coding exons and exon-intron boundaries of these genes in 54 boys or men from 46 families with a history of cryptorchidism. Control subjects (200) included 120 males. None of the patients carried mutation(s) in *FGFR1*, *PROK2*, *PROKR2*, *TAC3* or *TACR3*. Two of the 46 index subjects with unilateral cryptorchidism were heterozygous carriers of a single *GNRHR* mutation (Q106R or R262Q), also present in male controls with a similar frequency (3/120; $p = 0.62$). No homozygous or compound heterozygous *GNRHR* mutations were found. In conclusion, cryptorchidism is not commonly caused by defects in genes involved in IHH.

Keywords: Cryptorchidism, *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, *TACR3*

Abbreviations: *FGFR1*, fibroblast growth factor receptor 1; *GNRHR*, gonadotropin-releasing hormone receptor; *PROK2*, prokineticin 2; *PROKR2*, prokineticin receptor 2; *TAC3*, tachykinin 3; *TACR3*, tachykinin receptor 3; IHH, isolated hypogonadotropic hypogonadism; *AR*, androgen receptor; *INSL3*, insulin-like factor 3; *RXFP2*, relaxin/insulin-like family peptide receptor 2

1. Introduction

Cryptorchidism is one of the most common malformation in full-term boys with an estimated prevalence of 2-8% (Virtanen et al., 2007a), and an increasing incidence (Boisen et al., 2004). Testicular development and descent is a multifaceted process affected by anatomical, hormonal, environmental, and genetic factors (Damgaard et al., 2008; Foresta et al., 2008; Main et al., 2010; Skakkebaek et al., 2001; Virtanen et al., 2007a; Virtanen et al., 2007b). Mutations in the androgen receptor gene (*AR*) appear a rare cause of isolated congenital cryptorchidism (Ferlin et al., 2008). Recently, variation in the genes encoding insulin-like factor 3 (*INSL3*) that regulates the first, transabdominal phase of testicular descent (at least in mice) and in its receptor, *RFXP2* (*LGR8*), has been described in patients with congenital cryptorchidism (Ferlin et al., 2008; Foresta et al., 2008; Virtanen et al., 2007b). However, the reported role of these variants in the etiology of cryptorchidism is not consistent because of contradictory results (Ars et al., 2010; El Houate et al., 2008; Koskimies et al., 2000; Krausz et al., 2000; Lim et al., 2001; Nuti et al., 2008; Roh et al., 2003). Thus, relatively little is known about the genetic factors regulating testicular descent in humans.

Androgens have a pivotal role in testicular descent (Foresta et al., 2008). We have previously found reduced androgen bioactivity in 3-month-old boys with severe persistent cryptorchidism (Raivio et al., 2003), although immunoreactive testosterone levels did not show any differences between cases (all types included) and controls (Suomi et al., 2006). Expectedly, defects in the hypothalamic-pituitary-testicular (HPG) axis typically lead to cryptorchidism, a relatively common phenotypic feature in patients with isolated hypogonadotropic hypogonadism (IHH) (Pitteloud et al., 2002). It appears unlikely, however, that gene defects underlying IHH would account for isolated cryptorchidism, because these patients usually do not present with micropenis and may have slightly elevated rather than low gonadotropin levels during the minipuberty of infancy (Suomi et al., 2006). On the other hand, some IHH patients carrying mutation(s) in *FGFR1*, *GNRHR*, *PROK2/PROKR2* or *TAC3/TACR3* have displayed recovery of their hypogonadotropism (Gianetti et al., 2010; Pitteloud et al., 2005; Raivio et al., 2007; Sinisi et al., 2008), a phenomenon described also in a pediatric patient with an *FGFR1* mutation: this proband had bilateral cryptorchidism without

micropenis, but underwent spontaneous puberty as a teenager (Zenaty et al., 2006). It is currently unknown whether such reversal of hypogonadotropism could occur earlier, already during the first few months of life.

Very recently, Caronia et al. (2011) found six heterozygous rare variants in genes underlying IHH (two in *FGFR1* (G260E and R765H), two in *PROKR2* (R85H and L173R), one in *GNRHR* (R262Q), and one in *KALI* (V371I)) in women with hypothalamic amenorrhea, and suggested that these variants may cause at least partial susceptibility to HPG axis disorders. We recently reported a relatively high carrier frequency of two *GNRHR* mutations (Q106R and R262Q), both leading to partial inactivation of the receptor (de Roux et al., 1997; Layman et al., 1998), in Finnish population (altogether 5/200; 2.5%) (Vaaralahti et al., 2011). We therefore hypothesized that some patients with homozygous or compound heterozygous *GNRHR* mutations, or with rare variants in other genes underlying IHH, could present with cryptorchidism as a phenotypic sign of partially deficient GnRH signaling.

We investigated 54 Finnish boys or men from 46 families with a history of cryptorchidism for mutations in *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, and *TACR3*. The roles of *INSL3* and *RFXP2* in cryptorchidism have been previously studied in Finnish population, but only common polymorphisms were found with similar frequencies in cases and controls (Koskimies et al., 2000; Roh et al., 2003).

2. Subjects and Methods

2.1. Subjects

Altogether 54 subjects were included from 46 families. Of these 54 subjects, 13 had bi- and 39 had unilateral cryptorchidism, and information on the type of cryptorchidism in 2 cases was unavailable. Familial form of cryptorchidism, i.e. a cryptorchid first degree relative, was reported in 19 of the 46 participating families. Of the 54 subjects, 34 boys were followed up from birth and 33 of them had cryptorchidism diagnosed at birth (in preterm cases the diagnosis was verified at the expected date of delivery), and one boy was diagnosed at the age of 18 months. Boys were clinically examined after birth and at a mean age of 3 months, and were followed up until the age of 18 months. None of them had micropenis. The familial cases included 5 boys with bilateral (two of them were brothers) and 4 with unilateral cryptorchidism. Of the 34 subjects followed

up from birth, 19 boys of whom we had hormonal data at minipuberty all had normal gonadotropin and sex steroid levels during the minipuberty of infancy (Suomi et al., 2006) and puberty has started in 16 boys. Of the remaining 15/34 boys, 12 have by far displayed normal onset of puberty excluding classical IHH. Six boys, of whom 3 had normal hormone levels at minipuberty, are still too young to determine the onset of puberty, but they are being actively followed up. During the course of the follow-up, spontaneous descent of the testis was observed in 12 of the 34 boys.

In addition, 13 families including 20 male subjects participated via study on the familial incidence of cryptorchidism. Six of these male subjects reported of having congenital cryptorchidism. Of the 20 males, 19 had been previously treated for cryptorchidism, and one boy had had a spontaneous recovery of cryptorchidism. None of them displayed penile anomalies in infancy. In two families the fathers had unilateral and the sons had bilateral cryptorchidism; in three families, both the fathers and the sons had unilateral cryptorchidism; in one family, both brothers had unilateral cryptorchidism; and, in one family, the son had bilateral cryptorchidism and the type of the father's cryptorchidism remained unknown. In addition, three male subjects with unilateral cryptorchidism had a cryptorchid first degree relative. Unfortunately, the presence or absence of hypogonadism and the data on pubertal development of these subjects and their family members were unavailable. In one family, however, the pregnancy had been induced by microinjection, and both son and the father had cryptorchidism and were included in this study. No assisted reproductive techniques were used in 10 families, and in 2 families this information was unavailable.

Two hundred (120 males, 80 females) control subjects were included from the same geographical region. DNA was obtained from a blood sample. The study was accepted by the joint ethics committee of Turku University and Turku University Hospital, and informed consent was obtained from the participants or guardians of the participating boys.

2.2. Mutation screening of *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, and *TACR3*

Genomic DNA from peripheral blood leukocytes of the subjects was extracted, and the coding exons and exon-intron boundaries of *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, and *TACR3* were PCR amplified and screened by direct sequencing. In addition, exons 1-3 of the *GNRHR* were screened in control DNA in 200

(120 males, 80 females) subjects. PCR products were purified with ExoSAP-IT treatment (Amersham Biosciences, Piscataway, NJ, USA), and sequenced using the ABI BigDyeTerminator Cycle Sequencing Kit (v3.1) and ABI Prism 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Foster City, CA, USA). The sequences were aligned and read with Sequencher® 4.9 software (Gene Codes Corporation, Ann Arbor, MI, USA). All primer sequences and PCR conditions are available upon request.

2.3. Statistical analysis

All p -values are two-sided, and $p < 0.05$ was considered significant. Fisher's exact test was used to determine statistical significance between cases and controls (Simple Interactive Statistical Analysis, SISA, <http://www.quantitativeskills.com/sisa/>).

3. Results

3.1. Mutation screening of *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, and *TACR3*

We investigated 54 subjects with a history of cryptorchidism from 46 families for mutations in the *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, and *TACR3*. We did not identify mutations in the coding regions of *FGFR1*, *PROK2*, *PROKR2*, *TAC3*, or *TACR3*. However, two unrelated subjects, both with a positive family history of cryptorchidism in a first-degree relative, carried a heterozygous missense mutation, Q106R or R262Q, in *GNRHR*. Both subjects had unilateral cryptorchidism and neither of them had a spontaneous descent of a testis. The subject with R262Q mutation and his parents had normal onset of puberty, but the father had been operated for cryptorchidism. The second subject carrying a Q106R mutation had normal fertility, and his son had congenital cryptorchidism. Unfortunately, affected family members of these two subjects were unavailable for the analysis. These heterozygous missense *GNRHR* mutations were also present in 2 and 3 of 200 controls (2 and 1 of 120 males), respectively. Thus, the prevalence of the missense mutations between 46 index subjects and male controls did not differ ($p = 0.62$).

4. Discussion

We hypothesized that some patients with isolated cryptorchidism might harbor defects in genes previously shown to be indispensable for the intact function of the hypothalamic-pituitary-gonadal axis. Recently, Sykiotis et al. (2010) showed that some patients with IHH also have testicular defects, and Hadziselimovic et al. (2010) reported reduced expression of *FGFR1* in cryptorchid testis as compared to normally descended testis analyzed in testicular biopsies. Carriers of *FGFR1* mutations may have wide a phenotypic spectrum ranging from healthy carriers to severe IHH and Kallmann syndrome (IHH and decreased/absent sense of smell) (Dode et al., 2003; Pitteloud et al., 2005). However, none of the boys in the current study displayed mutations in the coding regions of *FGFR1*, suggesting that cryptorchidism is not commonly caused by IHH-causing *FGFR1* mutations with incomplete penetrance. Because we used DNA obtained from peripheral blood leukocytes, we cannot formally rule out, however, the possibility of tissue mosaicism of *FGFR1* mutations, previously described in a family with an IHH proband (Sato et al., 2006).

Two subjects with cryptorchidism carried a heterozygous missense change, Q106R or R262Q, in *GNRHR*. These mutations have previously been described in homozygous or compound heterozygous state in patients with IHH, delayed puberty, or with IHH but spontaneous pregnancy (Beranova et al., 2001; Caron et al., 1999; de Roux et al., 1997; Dewailly et al., 2002; Lin et al., 2006), whereas heterozygous carriers of these mutations were unaffected (Beranova et al., 2001; de Roux et al., 1997). On the other hand, R262Q was recently found in heterozygous state in a woman with hypothalamic amenorrhea (Caronia et al., 2011). However, we found that the frequency of heterozygous Q106R or R262Q *GNRHR* mutations did not differ between cases and male controls, and we therefore conclude that these heterozygous *GNRHR* mutations do not contribute to the prevalence of common cryptorchidism.

Neurokinin B and prokineticin signaling appears indispensable for HPG axis function in boys during late gestation and minipuberty of infancy, as evidenced by high frequency of micropenis among male IHH probands with *PROK2*, *PROKR2*, *TAC3*, or *TACR3* mutations (Abreu et al., 2008; Gianetti et al., 2010; Sarfati et al., 2010; Topaloglu et al., 2009). Since none of our subjects had micropenis or reported penile

anomalies, it was somewhat predictable that none of the subjects carried mutations in *TAC3*, *TACR3*, *PROK2*, or *PROKR2*.

The limitation of this study is a relatively small sample size. However, if mutation(s) in *FGFR1*, *GNRHR*, *TAC3*, *TACR3*, *PROK2*, or *PROKR2* did cause 10% of cryptorchidism, we would have with 99% probability found at least one case with conclusive mutation(s) among 46 index subjects. Similarly, if the prevalence of the mutation(s) was 4%, the probability to detect at least one is still 85%. Finally, the familial occurrence of cryptorchidism supports the genetic etiology for the condition (Weidner et al., 1999), and the risk for cryptorchidism in male infant is increased if a brother or father is affected (Elert et al., 2003; Foresta et al., 2008). In this study, 41% (19/46) of participating families had more than one cryptorchid family member, but high familial incidence herein was due to specific selection of the familial cases.

In conclusion, our results show that IHH-causing *FGFR1* mutations with incomplete penetrance, or homozygous/compound heterozygous *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, or *TACR3* mutations are not frequently encountered in patients with isolated cryptorchidism. It is therefore unlikely that mutations in these genes would contribute significantly to the prevalence of isolated cryptorchidism. Despite this, the familial cases suggest that there is a genetic susceptibility to common cryptorchidism.

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References

- 177 Abreu, A.P., Trarbach, E.B., de Castro, M., Frade Costa, E.M., Versiani, B., Matias Baptista, M.T., Garmes,
178 H.M., Mendonca, B.B., Latronico, A.C., 2008. Loss-of-function mutations in the genes encoding
179 prokineticin-2 or prokineticin receptor-2 cause autosomal recessive Kallmann syndrome. *J. Clin. Endocrinol.*
180 *Metab.* 93, 4113-4118.
- 181 Ars, E., Lo Giacco, D., Bassas, L., Nuti, F., Rajmil, O., Ruiz, P., Garat, J.M., Ruiz-Castane, E., Krausz, C.,
182 2010. Further insights into the role of T222P variant of RXFP2 in non-syndromic cryptorchidism in two
183 Mediterranean populations. *Int. J. Androl.* DOI: 10.1111/j.1365-2605.2010.01088.x.
- 184 Beranova, M., Oliveira, L.M., Bedecarrats, G.Y., Schipani, E., Vallejo, M., Ammini, A.C., Quintos, J.B.,
185 Hall, J.E., Martin, K.A., Hayes, F.J., Pitteloud, N., Kaiser, U.B., Crowley, W.F., Jr, Seminara, S.B., 2001.
186 Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor
187 mutations in idiopathic hypogonadotropic hypogonadism. *J. Clin. Endocrinol. Metab.* 86, 1580-1588.
- 188 Boisen, K.A., Kaleva, M., Main, K.M., Virtanen, H.E., Haavisto, A.M., Schmidt, I.M., Chellakooty, M.,
189 Damgaard, I.N., Mau, C., Reunanen, M., Skakkebaek, N.E., Toppari, J., 2004. Difference in prevalence of
190 congenital cryptorchidism in infants between two Nordic countries. *Lancet* 363, 1264-1269.
- 191 Caron, P., Chauvin, S., Christin-Maitre, S., Bennet, A., Lahlou, N., Counis, R., Bouchard, P., Kottler, M.L.,
192 1999. Resistance of hypogonadic patients with mutated GnRH receptor genes to pulsatile GnRH
193 administration. *J. Clin. Endocrinol. Metab.* 84, 990-996.
- 194 Caronia, L.M., Martin, C., Welt, C.K., Sykiotis, G.P., Quinton, R., Thambundit, A., Avbelj, M.,
195 Dhruvakumar, S., Plummer, L., Hughes, V.A., Seminara, S.B., Boepple, P.A., Sidis, Y., Crowley, W.F., Jr,
196 Martin, K.A., Hall, J.E., Pitteloud, N., 2011. A genetic basis for functional hypothalamic amenorrhea. *N.*
197 *Engl. J. Med.* 364, 215-225.
- 198 Damgaard, I.N., Jensen, T.K., Nordic Cryptorchidism Study Group, Petersen, J.H., Skakkebaek, N.E.,
199 Toppari, J., Main, K.M., 2008. Risk factors for congenital cryptorchidism in a prospective birth cohort study.
200 *PLoS One* 3, e3051.

- 201 de Roux, N., Young, J., Misrahi, M., Genet, R., Chanson, P., Schaison, G., Milgrom, E., 1997. A family with
202 hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N. Engl. J.*
203 *Med.* 337, 1597-1602.
- 204 Dewailly, D., Boucher, A., Decanter, C., Lagarde, J.P., Counis, R., Kottler, M.L., 2002. Spontaneous
205 pregnancy in a patient who was homozygous for the Q106R mutation in the gonadotropin-releasing hormone
206 receptor gene. *Fertil. Steril.* 77, 1288-1291.
- 207 Dode, C., Levilliers, J., Dupont, J.M., De Paepe, A., Le Du, N., Soussi-Yanicostas, N., Coimbra, R.S.,
208 Delmaghani, S., Compain-Nouaille, S., Baverel, F., Pecheux, C., Le Tessier, D., Cruaud, C., Delpech, M.,
209 Speleman, F., Vermeulen, S., Amalfitano, A., Bachelot, Y., Bouchard, P., Cabrol, S., Carel, J.C., Delemarre-
210 van de Waal, H., Goulet-Salmon, B., Kottler, M.L., Richard, O., Sanchez-Franco, F., Saura, R., Young, J.,
211 Petit, C., Hardelin, J.P., 2003. Loss-of-function mutations in *FGFR1* cause autosomal dominant Kallmann
212 syndrome. *Nat. Genet.* 33, 463-465.
- 213 El Houate, B., Rouba, H., Imken, L., Sibai, H., Chafik, A., Boulouiz, R., Chadli, E., Hassar, M., McElreavey,
214 K., Barakat, A., 2008. No association between T222P/LGR8 mutation and cryptorchidism in the Moroccan
215 population. *Horm. Res.* 70, 236-239.
- 216 Elert, A., Jahn, K., Heidenreich, A., Hofmann, R., 2003. The familial undescended testis. *Klin. Padiatr.* 215,
217 40-45.
- 218 Ferlin, A., Zuccarello, D., Zuccarello, B., Chirico, M.R., Zanon, G.F., Foresta, C., 2008. Genetic alterations
219 associated with cryptorchidism. *JAMA* 300, 2271-2276.
- 220 Foresta, C., Zuccarello, D., Garolla, A., Ferlin, A., 2008. Role of hormones, genes, and environment in
221 human cryptorchidism. *Endocr. Rev.* 29, 560-580.
- 222 Gianetti, E., Tusset, C., Noel, S.D., Au, M.G., Dwyer, A.A., Hughes, V.A., Abreu, A.P., Carroll, J.,
223 Trarbach, E., Silveira, L.F., Costa, E.M., de Mendonca, B.B., de Castro, M., Lofrano, A., Hall, J.E., Bolu, E.,
224 Ozata, M., Quinton, R., Amory, J.K., Stewart, S.E., Arlt, W., Cole, T.R., Crowley, W.F., Kaiser, U.B.,

- 225 Latronico, A.C., Seminara, S.B., 2010. TAC3/TACR3 mutations reveal preferential activation of
226 gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood.
227 J. Clin. Endocrinol. Metab. 95, 2857-2867.
- 228 Hadziselimovic, N.O., de Geyter, C., Demougin, P., Oakeley, E.J., Hadziselimovic, F., 2010. Decreased
229 expression of FGFR1, SOS1, RAF1 genes in cryptorchidism. Urol. Int. 84, 353-361.
- 230 Koskimies, P., Virtanen, H., Lindstrom, M., Kaleva, M., Poutanen, M., Huhtaniemi, I., Toppari, J., 2000. A
231 common polymorphism in the human relaxin-like factor (RLF) gene: no relationship with cryptorchidism.
232 Pediatr. Res. 47, 538-541.
- 233 Krausz, C., Quintana-Murci, L., Fellous, M., Siffroi, J.P., McElreavey, K., 2000. Absence of mutations
234 involving the INSL3 gene in human idiopathic cryptorchidism. Mol. Hum. Reprod. 6, 298-302.
- 235 Layman, L.C., Cohen, D.P., Jin, M., Xie, J., Li, Z., Reindollar, R.H., Bolbolan, S., Bick, D.P., Sherins, R.R.,
236 Duck, L.W., Musgrove, L.C., Sellers, J.C., Neill, J.D., 1998. Mutations in gonadotropin-releasing hormone
237 receptor gene cause hypogonadotropic hypogonadism. Nat. Genet. 18, 14-15.
- 238 Lim, H.N., Raipert-de Meyts, E., Skakkebaek, N.E., Hawkins, J.R., Hughes, I.A., 2001. Genetic analysis of
239 the INSL3 gene in patients with maldescent of the testis. Eur. J. Endocrinol. 144, 129-137.
- 240 Lin, L., Conway, G.S., Hill, N.R., Dattani, M.T., Hindmarsh, P.C., Achermann, J.C., 2006. A homozygous
241 R262Q mutation in the gonadotropin-releasing hormone receptor presenting as constitutional delay of
242 growth and puberty with subsequent borderline oligospermia. J. Clin. Endocrinol. Metab. 91, 5117-5121.
- 243 Main, K.M., Skakkebaek, N.E., Virtanen, H.E., Toppari, J., 2010. Genital anomalies in boys and the
244 environment. Best Pract. Res. Clin. Endocrinol. Metab. 24, 279-289.
- 245 Nuti, F., Marinari, E., Erdei, E., El-Hamshari, M., Echavarria, M.G., Ars, E., Balercia, G., Merksz, M.,
246 Giachini, C., Shaeer, K.Z., Forti, G., Ruiz-Castane, E., Krausz, C., 2008. The leucine-rich repeat-containing

- 247 G protein-coupled receptor 8 gene T222P mutation does not cause cryptorchidism. *J. Clin. Endocrinol.*
248 *Metab.* 93, 1072-1076.
- 249 Pitteloud, N., Acierno, J.S., Jr, Meysing, A.U., Dwyer, A.A., Hayes, F.J., Crowley, W.F., Jr, 2005. Reversible
250 kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in
251 the fibroblast growth factor receptor 1 gene. *J. Clin. Endocrinol. Metab.* 90, 1317-1322.
- 252 Pitteloud, N., Hayes, F.J., Boepple, P.A., DeCruz, S., Seminara, S.B., MacLaughlin, D.T., Crowley, W.F., Jr,
253 2002. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in
254 elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J. Clin. Endocrinol.*
255 *Metab.* 87, 152-160.
- 256 Raivio, T., Falardeau, J., Dwyer, A., Quinton, R., Hayes, F.J., Hughes, V.A., Cole, L.W., Pearce, S.H., Lee,
257 H., Boepple, P., Crowley, W.F., Jr, Pitteloud, N., 2007. Reversal of idiopathic hypogonadotropic
258 hypogonadism. *N. Engl. J. Med.* 357, 863-873.
- 259 Raivio, T., Toppari, J., Kaleva, M., Virtanen, H., Haavisto, A.M., Dunkel, L., Janne, O.A., 2003. Serum
260 androgen bioactivity in cryptorchid and noncryptorchid boys during the postnatal reproductive hormone
261 surge. *J. Clin. Endocrinol. Metab.* 88, 2597-2599.
- 262 Roh, J., Virtanen, H., Kumagai, J., Sudo, S., Kaleva, M., Toppari, J., Hsueh, A.J., 2003. Lack of LGR8 gene
263 mutation in Finnish patients with a family history of cryptorchidism. *Reprod. Biomed. Online* 7, 400-406.
- 264 Sarfati, J., Guiochon-Mantel, A., Rondard, P., Arnulf, I., Garcia-Pinero, A., Wolczynski, S., Brailly-Tabard,
265 S., Bidet, M., Ramos-Arroyo, M., Mathieu, M., Lienhardt-Roussie, A., Morgan, G., Turki, Z., Bremont, C.,
266 Lespinasse, J., Du Boullay, H., Chabbert-Buffet, N., Jacquemont, S., Reach, G., De Talence, N., Tonella, P.,
267 Conrad, B., Despert, F., Delobel, B., Brue, T., Bouvattier, C., Cabrol, S., Pugeat, M., Murat, A., Bouchard,
268 P., Hardelin, J.P., Dode, C., Young, J., 2010. A comparative phenotypic study of kallmann syndrome
269 patients carrying monoallelic and biallelic mutations in the prokineticin 2 or prokineticin receptor 2 genes. *J.*
270 *Clin. Endocrinol. Metab.* 95, 659-669.

- 271 Sato, N., Ohyama, K., Fukami, M., Okada, M., Ogata, T., 2006. Kallmann syndrome: somatic and germline
272 mutations of the fibroblast growth factor receptor 1 gene in a mother and the son. *J. Clin. Endocrinol. Metab.*
273 91, 1415-1418.
- 274 Sinisi, A.A., Asci, R., Bellastella, G., Maione, L., Esposito, D., Elefante, A., De Bellis, A., Bellastella, A.,
275 Iolascon, A., 2008. Homozygous mutation in the prokineticin-receptor2 gene (Val274Asp) presenting as
276 reversible Kallmann syndrome and persistent oligozoospermia: case report. *Hum. Reprod.* 23, 2380-2384.
- 277 Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M., 2001. Testicular dysgenesis syndrome: an
278 increasingly common developmental disorder with environmental aspects. *Hum. Reprod.* 16, 972-978.
- 279 Suomi, A.M., Main, K.M., Kaleva, M., Schmidt, I.M., Chellakooty, M., Virtanen, H.E., Boisen, K.A.,
280 Damgaard, I.N., Kai, C.M., Skakkebaek, N.E., Toppari, J., 2006. Hormonal changes in 3-month-old
281 cryptorchid boys. *J. Clin. Endocrinol. Metab.* 91, 953-958.
- 282 Sykiotis, G.P., Hoang, X.H., Avbelj, M., Hayes, F.J., Thambundit, A., Dwyer, A., Au, M., Plummer, L.,
283 Crowley, W.F., Jr, Pitteloud, N., 2010. Congenital idiopathic hypogonadotropic hypogonadism: evidence of
284 defects in the hypothalamus, pituitary, and testes. *J. Clin. Endocrinol. Metab.* 95, 3019-3027.
- 285 Topaloglu, A.K., Reimann, F., Guclu, M., Yalin, A.S., Kotan, L.D., Porter, K.M., Serin, A., Mungan, N.O.,
286 Cook, J.R., Ozbek, M.N., Imamoglu, S., Akalin, N.S., Yuksel, B., O'Rahilly, S., Semple, R.K., 2009. TAC3
287 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the
288 central control of reproduction. *Nat. Genet.* 41, 354-358.
- 289 Vaaralahti, K., Wehkalampi, K., Tammiska, J., Laitinen, E.M., Dunkel, L., Raivio, T., 2011. The role of
290 gene defects underlying isolated hypogonadotropic hypogonadism in patients with constitutional delay of
291 growth and puberty. *Fertil. Steril.* DOI: 10.1016/j.fertnstert.2010.12.059.
- 292 Virtanen, H.E., Bjerknes, R., Cortes, D., Jorgensen, N., Rajpert-De Meyts, E., Thorsson, A.V., Thorup, J.,
293 Main, K.M., 2007a. Cryptorchidism: classification, prevalence and long-term consequences. *Acta Paediatr.*
294 96, 611-616.

- 295 Virtanen, H.E., Cortes, D., Rajpert-De Meyts, E., Ritzen, E.M., Nordenskjold, A., Skakkebaek, N.E.,
296 Toppari, J., 2007b. Development and descent of the testis in relation to cryptorchidism. *Acta Paediatr.* 96,
297 622-627.
- 298 Weidner, I.S., Moller, H., Jensen, T.K., Skakkebaek, N.E., 1999. Risk factors for cryptorchidism and
299 hypospadias. *J. Urol.* 161, 1606-1609.
- 300 Zenaty, D., Bretones, P., Lambe, C., Guemas, I., David, M., Leger, J., de Roux, N., 2006. Paediatric
301 phenotype of Kallmann syndrome due to mutations of fibroblast growth factor receptor 1 (FGFR1). *Mol.*
302 *Cell. Endocrinol.* 254-255, 78-83.
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305 **Highlights**

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- 307 • We investigated the defects in *FGFR1*, *GNRHR*, *PROK2*, *PROKR2*, *TAC3*, and *TACR3* in 54
308 subjects with isolated cryptorchidism.
- 309 • Two subjects carried heterozygous *GNRHR* mutations (Q106R and R262Q), but these changes
310 were also present in controls with similar frequency.
- 311 • None of the subjects carried mutation(s) in *FGFR1*, *PROK2*, *PROKR2*, *TAC3* or *TACR3*.
- 312 • It is unlikely that mutations in these genes would significantly contribute to the prevalence of
313 isolated cryptorchidism.

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