Novel *FGFR1* mutations in Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism: evidence for the involvement of an alternatively spliced isoform

Catarina Gonçalves, M.Sc.,[a] Margarida Bastos, M.D.,[b] Duarte Pignatelli, M.D., Ph.D.,[c] Teresa Borges, M.D.,[d] José M. Aragüés, M.D.,[e] Fernando Fonseca, M.D.,[f] Bernardo D. Pereira, M.D.,[g] Sílvia Socorro, Ph.D.,[a] and Manuel C. Lemos, M.D., Ph.D.[a]

[a] CICS-UBI, Health Sciences Research Centre, University of Beira Interior, Covilhã; [b] Serviço de Endocrinologia, Diabetes e Metabolismo, Centro Hospitalar Universitário de Coimbra, Coimbra; [c] Serviço de Endocrinologia, Diabetes e Metabolismo, Hospital de São João e Faculdade de Medicina do Porto; [d] Serviço de Pediatria Médica, Centro Hospitalar do Porto, Porto; [e] Serviço de Endocrinologia, Diabetes e Metabolismo, Hospital de Santa Maria; [f] Serviço de Endocrinologia, Diabetes e Metabolismo, Hospital de Curry Cabral, Lisbon; and [g] Serviço de Endocrinologia e Diabetes, Hospital Garcia de Orta, Almada, Portugal

Objective: To determine the prevalence of fibroblast growth factor receptor 1 (*FGFR1*) mutations and their predicted functional consequences in patients with idiopathic hypogonadotropic hypogonadism (IHH).
Design: Cross-sectional study.
Setting: Multicentric.
Patient(s): Fifty unrelated patients with IHH (21 with Kallmann syndrome and 29 with normosmic IHH).
Intervention(s): None.
Main Outcome Measure(s): Patients were screened for mutations in *FGFR1*. The functional consequences of mutations were predicted by in silico structural and conservation analysis.
Result(s): Heterozygous *FGFR1* mutations were identified in six (12%) kindreds. These consisted of frameshift mutations (p.Pro33-Alafs*17 and p.Tyr654*) and missense mutations in the signal peptide (p.Trp4Cys), in the D1 extracellular domain (p.Ser96Cys) and in the cytoplasmic tyrosine kinase domain (p.Met719Val). A missense mutation was identified in the alternatively spliced exon 8A (p.Ala353Thr) that exclusively affects the D3 extracellular domain of FGFR1 isoform IIIb. Structure-based and sequence-based prediction methods and the absence of these variants in 200 normal controls were all consistent with a critical role for the mutations in the activity of the receptor. Oligogenic inheritance (*FGFR1/CHD7/PROKR2*) was found in one patient.
Conclusion(s): Two FGFR1 isoforms, IIIb and IIIc, result from alternative splicing of exons 8A and 8B, respectively. Loss-of-function of isoform IIIc is a cause of IHH, whereas isoform IIIb is thought to be redundant. Ours is the first report of normosmic IHH associated with a mutation in the alternatively spliced exon 8A and suggests that this disorder can be caused by defects in either of the two alternatively spliced FGFR1 isoforms. (Fertil Steril® 2015;104: 1261–7. ©2015 by American Society for Reproductive Medicine.)
Key Words: Hypogonadotropic hypogonadism, Kallmann syndrome, FGFR1, KAL2, genetics

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/novel-fgfr1-mutations-ihh/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received March 30, 2015; revised July 19, 2015; accepted July 20, 2015; published online August 12, 2015.
C.G. reports grants from Portuguese Foundation for Science and Technology. M.B. has nothing to disclose. D.P. has nothing to disclose. T.B. has nothing to disclose. J.M.A. has nothing to disclose. F.F. has nothing to disclose. B.D.P. has nothing to disclose. S.S. reports grants from Portuguese Foundation for Science and Technology. M.C.L. reports grants from Portuguese Foundation for Science and Technology.
Supported by the Portuguese Foundation for Science and Technology (grant PTDC/SAU-GMG/098419/2008). C.G. is the recipient of a Ph.D. fellowship grant (SFRH/BD/76420/2011). D.P. and M.C.L. are national representatives for Portugal in the European Cooperation in Science and Technology (COST) Action BM1105 "GnRH Deficiency: Elucidation of the Neuroendocrine Control of Human Reproduction."
Reprint requests: Manuel C. Lemos, M.D., Ph.D., CICS-UBI, Health Sciences Research Centre, University of Beira Interior, 6200-506 Covilhã, Portugal (E-mail: mclemos@fcsaude.ubi.pt).

Fertility and Sterility® Vol. 104, No. 5, November 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.07.1142

Idiopathic hypogonadotropic hypogonadism (IHH) is defined by complete or partial failure of pubertal development due to the compromised secretion of gonadotropins (FSH and LH) and sex hormones (testosterone [T] and estrogens [E]), in the absence of any hypothalamic-pituitary organic cause (1). Congenital forms of IHH include Kallmann syndrome (KS), which is characterized by gonadotropin deficiency with a defective sense of smell (anosmia or hyposmia), and IHH without olfactory defects (normosmic IHH) (2). In addition, nonreproductive phenotypes, such as midline facial defects, dental agenesis, hearing loss, renal agenesis, synkinesis, and digital bone abnormalities, are commonly observed in patients with IHH (2, 3). About one third of patients with IHH reveal a genetic defect in genes that regulate the embryonic development or migration of GnRH neurons, or the synthesis, secretion, or action of GnRH (4, 5). One of the most frequently implicated genes is the fibroblast growth factor receptor 1 (*FGFR1*, OMIM 136350) gene, which is located at chromosome 8p11.2, and comprises 18 coding exons (6, 7). At present, 219 loss-of-function *FGFR1* mutations have been associated with autosomal dominant forms of KS and normosmic IHH (Supplemental Table 1, available online).

The FGFR1 protein is a transmembrane receptor that comprises an extracellular region of three immunoglobulin-like domains (D1, D2, and D3), a transmembrane helix, and a cytoplasmic tyrosine kinase domain (8). Alternative splicing of the carboxy-terminal half of D3, through the use of either exon 8A or 8B, generates isoforms FGFR1-IIIb or FGFR1-IIIc, respectively (9). Although these isoforms have different tissue expression and FGF-binding affinity (10), experimental data suggest that FGFR1-IIIc is the dominant isoform that carries out most of the biological functions of the *FGFR1* gene, whereas IIIb plays a minor and somewhat redundant role (11).

The aim of this study was to identify and determine the prevalence of *FGFR1* mutations in a cohort of Portuguese patients with KS and normosmic IHH, and to investigate the consequences of these mutations.

MATERIALS AND METHODS

Subjects

This cross-sectional study comprised 50 unrelated Portuguese patients with IHH (43 men and 7 women), 21 with KS and 29 with normosmic IHH, recruited by Portuguese clinical endocrine centers from 2010 to 2014. Inclusion criteria were male and female patients with IHH, with failure to enter spontaneous puberty by the age of 18 years, or with medically induced puberty at a younger age, or with documented anosmia. Patients with a history of an acquired cause of hypopituitarism were excluded from the study. Whenever possible, unaffected family members were also studied. The control population consisted of 200 Portuguese unrelated volunteers who were recruited among blood donors. Written informed consent was obtained from all subjects and the study was approved by the local research ethics committee (Faculty of Health Sciences, University of Beira Interior; CE-FCS-2012-012).

Genetic Studies

Genomic DNA was extracted from peripheral blood leukocytes using previously described methods (12). Patients were screened for mutations in *FGFR1* by polymerase chain reaction (PCR) amplification of the 18 coding exons and exon–intron boundaries, and bidirectional sequencing using a CEQ DTCS sequencing kit (Beckman Coulter) and an automated capillary DNA sequencer (GenomeLab TM GeXP, Genetic Analysis System; Beckman Coulter). Primer sequences were previously described by Sato et al. (13), except for primers for exons 14 and 15 that were described by Albuisson et al. (14). Heterozygous frameshift mutations were confirmed by cloning of the PCR products using pGEM-T Easy Vector Systems (Promega Corporation), followed by DNA sequencing of each allele. Mutations were confirmed in patients and excluded in a panel of 200 healthy volunteers (400 alleles) using sequence-specific restriction enzymes. The mutation in exon 14 did not create or eliminate any restriction enzyme recognition site, therefore a restriction site was introduced on the mutated allele using a modified PCR forward primer (5′-GACATTCACCACATCGACTATTA-3′) (modified nucleotide underlined). The same occurred for the mutation in exon 2, thereby a restriction site was introduced on the wild-type allele using a modified PCR forward primer (5′-AGAACTGGGATGTGGAACTG-3′) (modified nucleotide underlined). Mutation nomenclature followed standard guidelines (15) and was based on the complementary DNA (cDNA) reference sequence for the FGFR1-IIIc isoform (GenBank accession NM_023110.2) or the FGFR1-IIIb isoform (GenBank accession FJ809917) (in the case of mutation in the alternatively spliced exon 8A). Patients with identified *FGFR1* mutations were screened for digenic/oligogenic mutations by sequencing additional genes related to the hypothalamic-pituitary-gonadal axis (*KAL1*, *GNRH1*, *GNRHR*, *FGF8*, *PROK2*, *PROKR2*, *KISS1R*, *TAC3*, *TACR3*, *NELF*, and *CHD7*) (all primer sequences and PCR conditions are available upon request).

In Silico Structural and Conservation Analysis

The functional consequences of the observed missense mutations were predicted by the use of different bioinformatic tools: SIFT (16), Provean (17), PolyPhen 2.0 (18), and Mutation Taster (19). The mapping of the mutations onto the known FGFR1 crystal structure was carried out using the PyMol Molecular Graphics System (20). The conservation analysis of mutated amino acids across species was performed using the Mutation Taster software (19). Mutations that resulted in frameshifts did not lead to any other further studies, as they could be considered pathogenic due to their highly disruptive effect on protein structure or expression.

RESULTS

FGFR1 Mutations and Associated Clinical Characteristics

Sequence analysis of the entire coding region of *FGFR1*, including exon–intron boundary regions, revealed six novel heterozygous mutations: two frameshift (c.95dupA