

Clinical Genetic Testing for Kallmann Syndrome

Lawrence C. Layman

Section of Reproductive Endocrinology, Infertility, and Genetics, Department of Obstetrics and Gynecology, Institute of Molecular Medicine and Genetics, Neuroscience Program, Medical College of Georgia, Georgia Regents University, Augusta, Georgia 30912

During the past 20 years, remarkable advances have been made regarding the molecular basis of many disorders affecting reproduction. This is particularly true for patients who present with delayed puberty due to GnRH deficiency. These affected males and females manifest low serum levels of sex steroids, low or normal gonadotropins, and no other pituitary pathology. The pathophysiology of GnRH deficiency is complex, but 2 major phenotypes are observed. When hypothalamic GnRH gene regulation or GnRH synthesis, secretion, or signaling is impaired, the resulting phenotype is normosmic hypogonadotropic hypogonadism (nHH). However, it is also known that GnRH neurons originate outside of the brain and migrate along with olfactory neurons from the nasal region into the hypothalamus. If this migratory pathway of GnRH and olfactory neurons is disrupted, anosmia accompanies hypogonadotropic hypogonadism, known as Kallmann syndrome (KS).

KAL1, the first gene for KS, was identified more than 20 years ago in affected males with X-linked recessive KS. Valuable families with Xp deletions and chromosomal translocations permitted the localization of this gene on chromosome Xp22.32 (1, 2). Confirmation that *KAL1* mutations cause KS was obtained by the demonstration of intragenic deletions and point mutations segregating within families, which were absent in unaffected controls (3, 4). About 7 years later, mutations in *GNRHR* (GnRH receptor) were identified in nHH patients (5, 6). The *GNRHR* gene was the first gene in which mutations were found to cause nHH and autosomal recessive disease. Therefore, *GNRHR* was the first causative gene identified to be involved in GnRH deficiency in women (5, 6).

Since that time, mutations in at least 16 other genes and 6 more causing combined pituitary hormone deficiency have been identified to cause nHH and KS in autosomal dominant, autosomal recessive, and X-linked recessive inheri-

tance patterns (7). Mutations in 2 (digenic) or more (oligogenic) genes have been reported in a small percentage of KS and nHH patients (8, 9). In addition, a variety of associated nonreproductive anomalies may be seen in KS patients such as unilateral renal agenesis, midline facial defects, dental agenesis, skeletal abnormalities, neurological abnormalities such as synkinesia, ataxia, or visual symptoms, deafness, and cardiac anomalies (7). Mutations in some genes seem to cause just KS (*KAL1*) or just nHH (such as ligand/receptor pairs *GNRH1/GNRHR*, *LEP/LEPR*, *KISS1/KISS1R*, and *TACR3/TAC3*; and rarely *NR0B1* or *PCSK1*), whereas some may cause either nHH or KS (*FGFR1*, *FGF8*, *CHD7*, *HS6ST1*, *PROK2/PROKR2*, *NELF*, *WDR11*, and *SEMA3A*) (7). Recently, mutations in the pituitary transcription factor *HESX1*, known to result in septo-optic dysplasia, combined pituitary hormone deficiency, and isolated GH deficiency, have also been found in patients with KS, illustrating overlap between apparent pituitary and hypothalamic disorders (10).

How should the clinician prioritize these genes to clinically evaluate KS patients that manifest these anomalies? The mode of inheritance will certainly be important in genetic counseling of these patients, not only for the risk of pubertal disorders in their children, but also for other potentially life-altering nonreproductive anomalies. This is exactly what was addressed in the study in this issue of the *JCEM* by Costa-Barbosa et al (11), who studied 219 KS patients (male and female) for 8 KS genes in 6 pathways (*KAL1*, *FGF8/FGFR1*, *PROK2/PROKR2*, *HS6ST1*, *NELF*, and *CHD7*). The investigators hypothesized that mutations in these 6 pathways would exhibit specific phenotypes that could be used for clinical genetic testing in KS patients. Of the 219 KS patients, 151 had rare sequence variants (RSVs) in at least 1 of these genes, whereas 68 had no RSV identified. RSVs were defined as

ISSN Print 0021-972X ISSN Online 1945-7197

Printed in U.S.A.

Copyright © 2013 by The Endocrine Society

Received March 11, 2013. Accepted March 14, 2013.

For article see page E943

Abbreviations: KS, Kallmann syndrome; nHH, normosmic hypogonadotropic hypogonadism; RSV, rare sequence variant.

DNA sequence changes that alter amino acids, predict protein truncation, or are intronic within 10 base pairs of splice junctions that occur in less than 1% of control populations. The term RSV is often used because mutations imply impaired function, but not all nucleotide changes identified in patients can be studied for function in vitro (and they were not in the present study).

These investigators compared the phenotypes for RSVs in 5 of the 6 pathways (there was not enough clinical information available for *NELF* RSVs), and they found the following:

Reproductive phenotype. The most severe phenotype with regard to the complete absence of puberty, and therefore, testes size, was found for males with *KAL1* mutations when compared with the other 4 pathway groups and the RSV-negative group. Severity was not different in these 5 pathways for female KS patients.

Unilateral renal agenesis. Unilateral renal agenesis has been thought to exclusively occur in *KAL1* mutations (7); it was also seen in the RSV-negative group, but not in any of the other 4 groups.

Synkinesia. Synkinesia is most often thought to be observed in *KAL1* and *FGFR1* genotypes (7) and was more often seen in *KAL1* vs non-*KAL1* probands. Interestingly, it was seen in all of the other groups, including the RSV-negative group, but not in *HS6ST1*.

Hearing loss. Hearing loss was more common in the *CHD7* vs non-*CHD7* group, but it was seen in all of the other groups except *HS6ST1*.

Cleft lip/palate. Cleft lip/palate is expected with *FGFR1/FGF8* and *CHD7* and was seen in all groups except the *KAL1* and *PROK2/PROKR2* groups.

Dental agenesis. Dental agenesis was seen most commonly in the *FGFR1/FGF8* group but was also identified in the *CHD7* and RSV-negative groups.

Skeletal anomalies. Only syndactyly, polydactyly, or camptodactyly were exclusively seen in the *FGFR1/FGF8* group (and none of the other groups, including the RSV-negative group). Other skeletal anomalies such as scoliosis, kyphosis, excessive joint mobility, short fourth metacarpal bones, clinodactyly, foreshortened limb bones, and flat feet were seen in all groups.

Several of these findings are particularly interesting. The identification of renal agenesis in the RSV-negative group is perhaps unexpected because it has been a common finding in males with *KAL1* mutations (12). As the authors suggest,

unidentified *KAL1* mutations outside of the coding and splice site regions (as in the promoter or deep into the intron) could partially account for this finding (because these gene regions were not tested), but it is also possible that genes yet to be discovered might contribute to renal development. It is also interesting that synkinesia was seen across most of the groups because it has been previously thought to occur with *KAL1* and *FGF8/FGFR1* mutations (7). This observation suggests that perhaps synkinesia occurs more often than is currently appreciated and should routinely be tested for when KS patients are examined.

It is not surprising that hearing loss is most common with *CHD7* mutations vs non-*CHD7* groups because mutations in this gene were first identified in CHARGE syndrome, which has ear abnormalities as a feature (13). CHARGE syndrome consists of coloboma of the eye, heart defects, atresia of the choanae (posterior nasopharyngeal obstruction), retardation of growth and development, genital abnormalities, and ear (auditory and/or vestibular dysfunction) anomalies. It was only later when KS was suspected to be a milder allelic variant of CHARGE syndrome that *CHD7* mutations were identified in KS and nHH patients without full CHARGE features (14). Interestingly though, hearing loss is present in all other gene groups except *HS6ST1*, which again attests to the importance of ascertaining hearing competence when examining KS patients. Skeletal anomalies were first described for patients with *FGFR1* mutations (15). In the present study (11), several skeletal abnormalities of the hands—syndactyly, polydactyly, or camptodactyly—were exclusively seen in the *FGF8/FGFR1* RSV group. Additionally, response to GnRH and the reversibility of hypogonadism were addressed, but the sample size was too small to make comparisons.

Based upon their findings, the authors propose that genetic testing can be prioritized upon associated nonreproductive phenotypes: synkinesia, *KAL1*; hearing loss, *CHD7*; digital bony defects and dental agenesis, *FGFR1/FGF8* (11). This collaborative group of investigators possesses a great number of KS patient samples and clinical information, which is important for studying genotype/phenotype correlations. However, even with a large sample size, the frequency of RSVs in the affected genotypes is low. Although the numbers in *KAL1* ($n = 38$), *FGF8/FGFR1* ($n = 54$), *PROK2/PROKR2* ($n = 31$), and *CHD7* ($n = 22$) groups are reasonable, the sample size is smaller for *HS6ST1* ($n = 4$) and the excluded *NELF* ($n = 2$) groups. This makes it more difficult to compare the groups for significant differences. Although only 109 of the 151 KS patients with RSVs had all 8 genes studied and digenic disease could have been missed in a small percentage of patients, this is unlikely to have significantly affected their findings (they did exclude known digenic disease unless they were part of a ligand/receptor pair,

such as *PROK2/PROKR2*). In addition, heterozygous *PROK2* and *PROKR2* RSVs were considered in this study (11), but the inheritance has been uncertain as to whether it is autosomal recessive or dominant (16, 17). Nevertheless, the findings by Costa-Barbosa et al (11) could form the basis for a database that will hopefully be expanded and contributed to internationally by other authors studying these disorders. This is perhaps the best way to collate this type of data, which will provide a valuable resource for patients and physicians.

What should the clinician do when confronted with the KS patient? A targeted approach of genetic testing can be performed as suggested by the authors. In addition, if the prevalence of gene mutations and/or RSVs is considered for KS, *FGFR1* (10%) and *CHD7* (6%) are the most common autosomal causes of KS, whereas *KAL1* has been estimated to have a prevalence of 5–10% of affected males (X-linked recessive) and much higher in the clearly delineated X-linked recessive pedigrees (18). Therefore, the clinician should probably consider these 3 genes in any KS male (and *FGFR1* and *CHD7* in KS females). Costa-Barbosa et al (11) did not address nHH in this study, but *FGFR1* and *CHD7* mutations occur at similar prevalences in this group, and uniquely nHH genes *GNRHR* (4%) and *TACR3* (5–6%) could be considered in this group. Targeted gene testing based upon the findings from this study is very reasonable, as is consideration for the most common genes that cause KS. It should be kept in mind that Sanger DNA sequencing (which would usually be done by clinical laboratories or research groups such as the authors' group or ours) will miss heterozygous intragenic deletions that are larger than the PCR amplicon. Therefore, testing for deletions/duplications using a technique such as multiplex ligation-dependent probe amplification will need to be performed for exclusion. This method was not used in the current study, and unless otherwise stated, this is also true for most genetic studies of human disease.

We are now in the middle of an unprecedented increase in high throughput and reduced cost in massively parallel next generation DNA sequencing (19). To sequence 3 to 5 of the most likely genes in a disorder such as KS will be replaced in the near future by targeted sequencing of all known causative genes. Because the cost is being reduced and the depth of coverage increased, sequencing all exons of all genes (whole exome sequencing) and all 3 billion bases in the genome (whole genome sequencing) will likely replace many of the clinical tests that are currently being performed. Until the bottleneck of bioinformatic analysis is overcome, targeted gene analysis for the most common and likely genes that cause KS will be beneficial to patients and physicians alike.

Acknowledgments

Address all correspondence and requests for reprints to: Lawrence C. Layman, MD, Section of Reproductive Endocrinology,

Infertility, and Genetics, Department of Obstetrics and Gynecology, Institute of Molecular Medicine and Genetics, Neuroscience Program, Medical College of Georgia, Georgia Regents University, 1120 15th Street, Augusta, Georgia 30912. E-mail: lalayman@gru.edu.

Funding was provided by National Institutes of Health Grant HD033004.

Disclosure Summary: The author has nothing to disclose.

References

1. Franco B, Guioli S, Pragliola A, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*. 1991;353:529–536.
2. Legouis R, Hardelin JP, Leveilliers J, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell*. 1991;67:423–435.
3. Bick D, Franco B, Sherins RS, et al. Intragenic deletion of the *KALIG-1* gene in Kallmann's syndrome. *N Engl J Med*. 1992;326:1752–1755.
4. Hardelin JP, Leveilliers J, del Castillo I, et al. X Chromosome-linked Kallmann syndrome: stop mutations validate the candidate gene. *Proc Natl Acad Sci USA*. 1992;89:8190–8194.
5. de Roux N, Young J, Misrahi M, et al. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med*. 1997;337:1597–1602.
6. Layman LC, Cohen DP, Jin M, et al. Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. *Nat Genet*. 1998;18:14–15.
7. Layman LC. The genetic basis of female reproductive disorders: etiology and clinical relevance [published online ahead of print March 11, 2013]. *Mol Cell Endocrinol*. doi: 10.1016/j.mce.2013.02.016.
8. Pitteloud N, Quinton R, Pearce S, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest*. 2007;117:457–463.
9. Quaynor SD, Kim HG, Cappello EM, et al. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertil Steril*. 2011;96:1424–1430 e1426.
10. Newbern K, Natrajan N, Kim HG, et al. Identification of *HESX1* mutations in Kallmann syndrome [published online ahead of print March 1, 2013]. *Fertil Steril*. doi: 10.1016/j.fertnstert.2013.01.149
11. Costa-Barbosa F, Balasubramanian R, Keefe KW, et al. Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotype. *J Clin Endocrinol Metab*. 2013;98:E943–953
12. Hardelin JP, Leveilliers J, Blanchard S, et al. Heterogeneity in the mutations responsible for X chromosome-linked Kallmann syndrome. *Hum Mol Genet*. 1993;2:373–377.
13. Vissers LE, van Ravenswaaij CM, Admiraal R, et al. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet*. 2004;36:955–957.
14. Kim HG, Kurth I, Lan F, et al. Mutations in *CHD7*, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet*. 2008;83:511–519.
15. Dode C, Leveilliers J, Dupont JM, et al. Loss-of-function mutations in *FGFR1* cause autosomal dominant Kallmann syndrome. *Nat Genet*. 2003;33:463–465.
16. Abreu AP, Trarbach EB, de Castro M, et al. Loss-of-function mutations in the genes encoding prokineticin-2 or prokineticin receptor-2 cause autosomal recessive Kallmann syndrome. *J Clin Endocrinol Metab*. 2008;93:4113–4118.
17. Dode C, Teixeira L, Leveilliers J, et al. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet*. 2006;2:e175.
18. Bhagavath B, Xu N, Ozata M, et al. *KAL1* mutations are not a common cause of idiopathic hypogonadotropic hypogonadism in humans. *Mol Hum Reprod*. 2007;13:165–170.
19. Biesecker LG. Exome sequencing makes medical genomics a reality. *Nat Genet*. 2010;42:13–14.