

of them showed only a very marginal association. This result should signal caution to groups that are hastily progressing to replication studies with only a handful of best-associated SNPs.

Finally, we should remember that, by definition, genomewide association studies that rely on common SNPs monitor only common alleles, but there is so much more in the genetic risk profiles behind common diseases such as multiple sclerosis. We need to define the full allelic diversity of the “suspicious genes” that are initially identified by genomewide studies. By sequencing the potential risk alleles in large study samples, we will probably encounter rare, high-impact alleles with critical importance for disease risk in some families or patients. The lessons learned from studies of genetic risk variants like *BRCA1* and *BRCA2*, which are rare alleles with a high impact but which explain only a small fraction of breast cancers, will be instructive for our thinking about other complex diseases. The somewhat disappointing outcome with respect to the attributable risk conferred by the SNPs sampled in *IL2RA* and *IL7RA* indicates that other types of genome variants should be sought. Without a doubt, the multiple sclerosis community will soon be informed about the systematic scans for copy-number variations or other more complex changes

in the genomic architecture of risk alleles for multiple sclerosis.

No potential conflict of interest relevant to this article was reported.

This article (10.1056/NEJMe078147) was published at www.nejm.org on July 29, 2007.

From the University of Helsinki and the National Public Health Institute, Biomedicum, Helsinki, Finland; and the Broad Institute, Massachusetts Institute of Technology, Cambridge, MA.

1. Ebers GC, Kukay K, Bulman DE, et al. A full genome search in multiple sclerosis. *Nat Genet* 1996;13:472-6.
2. Haines JL, Ter-Minassian M, Bazyk A, et al. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. *Nat Genet* 1996;13:469-71.
3. Sawcer S, Jones HB, Feakes R, et al. A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat Genet* 1996;13:464-8.
4. Kuokkanen S, Gschwend M, Rioux JD, et al. Genomewide scan of multiple sclerosis in Finnish multiplex families. *Am J Hum Genet* 1997;61:1379-87.
5. The International Multiple Sclerosis Genetics Consortium. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med* 2007;357:851-62.
6. Gregory SG, Schmidt S, Seth P, et al. Interleukin 7 receptor alpha chain shows allelic and functional association with multiple sclerosis. *Nat Genet* (in press).
7. Lundmark F, Duvefeldt K, Jacobaeus E, et al. Variation in interleukin-7 receptor alpha chain influences risk of multiple sclerosis. *Nat Genet* (in press).
8. Kuokkanen S, Sundvall M, Terwilliger JD, et al. A putative vulnerability locus to multiple sclerosis maps to 5p14-p12 in a region syntenic to the murine locus *Eae2*. *Nat Genet* 1996;13:477-80.

Copyright © 2007 Massachusetts Medical Society.

Experiments of Nature — A Glimpse into the Mysteries of the Pubertal Clock

Shalender Bhasin, M.D.

In an extraordinary display of nature's myriad intricacies, in higher mammals the gonadotropin-releasing hormone (GnRH) pulse generator, which drives the pulsatile secretion of gonadotropin and sex steroids, is kept in abeyance until the onset of puberty, when it is reactivated with remarkable predictability during the pubertal transition. Its role in this transition is to promote sexual maturation in synchrony with somatic growth and maturation of sexual and social behaviors. In an earlier era, when most humans died before their 25th birthday, food availability was precarious, and environmental conditions were unpredictable, failure of the reproductive axis to activate in a timely manner, or even at all, could

threaten reproductive potential and survival. In light of these selection pressures, it is not surprising that single-gene mutations associated with disordered gonadotropin secretion and action are uncommon.

Such mutations, although rare, have contributed greatly to our understanding of the regulation of gonadotropin secretion and action.^{1,2} Studies of patients with idiopathic hypogonadotropic hypogonadism have uncovered the important role of a number of genes in the development, migration, and networking of GnRH neurons and the regulation of GnRH secretion: *KAL1* (Kallmann syndrome 1 sequence 1), *FGFR1* (fibroblast growth factor receptor 1), *GNRHR*

(GnRH receptor), *GPR54* (G protein–coupled receptor 54), *LEP* (leptin) and its receptor SF-1 (steroidogenic factor 1), *DAX-1* (nuclear receptor subfamily 0, group B, member 1 [also known as *NROB1*]), and *NELF* (nasal embryonic luteinizing hormone–releasing hormone factor). This list of genes is not exhaustive, and the list continues to grow.^{2,3} Similarly, mutations of the beta subunits of luteinizing hormone and follicle-stimulating hormone, and the cognate receptors of these hormones, have provided unique insights into the role of these hormones in male and female reproduction.^{1,4} In this issue of the *Journal*, two genetic disorders of gonadotropin secretion are highlighted: a reversible form of congenital GnRH deficiency, in the article by Raivio et al.,⁵ and a new mutation in the luteinizing hormone beta-subunit gene (*LHB*), associated with anovulatory infertility in a woman, in the article by Lofrano-Porto et al.⁶ These reports further illustrate the important role of the GnRH–gonadotropin axis in sexual maturation and in the regulation of reproductive function in men and women.

The prevalent dogma assumes that idiopathic hypogonadotropic hypogonadism represents irreversible GnRH deficiency requiring lifelong therapy, although several reports^{5,7,8} suggest that a small percentage of men with idiopathic hypogonadotropic hypogonadism undergo a sustained reversal of hypogonadotropism. Thus, in a small subgroup of patients with idiopathic hypogonadotropic hypogonadism, activation of the GnRH–gonadotropin axis is markedly delayed. It is unclear whether this is a new or even a distinct disorder or whether this condition represents one extreme on a phenotypic spectrum of disorders characterized by delayed activation of the GnRH pulse generator.

There are wide variations in the onset of puberty; puberty is considered to be delayed if the initial signs of sexual maturation do not appear by an age that is 2.5 SD beyond the mean for healthy boys or girls.^{9,10} Myriad conditions can delay the onset of pubertal maturation; if no underlying condition can explain pubertal delay, and if sexual maturation occurs before the age of 18 years, a diagnosis of constitutional delay of growth and development is made.^{9,10} This diagnosis is established with certainty only after the patient displays unequivocal signs of pubertal transition, such as increasing testicular volume and rising testosterone concentrations; at initial presentation, these patients are indistinguishable

from those with idiopathic hypogonadotropic hypogonadism. Both conditions are characterized by a lack of timely activation of the GnRH–gonadotropin axis; if there are no signs of activation of the GnRH–gonadotropin axis by 18 years of age, we assume that the patient has idiopathic hypogonadotropic hypogonadism and that the hypogonadotropism is likely to persist throughout life.

In a systematic investigation of children with delayed puberty, Sedlmeyer and Palmert¹⁰ reported that the delay was constitutional in approximately two thirds of boys and one third of girls with delayed puberty; approximately 20% had functional hypogonadotropic hypogonadism (defined by the presence of an underlying condition associated with delayed pubertal development and hypogonadotropism), 12% had permanent idiopathic hypogonadotropic hypogonadism, 13% had hypergonadotropic hypogonadism, and a small percentage had no identifiable disorder. Patients with constitutional pubertal delay and functional hypogonadotropic hypogonadism eventually undergo spontaneous puberty¹⁰; the data reported by Raivio et al.⁵ indicate that some patients with idiopathic hypogonadotropic hypogonadism will also eventually have normal gonadotropin secretion.

Constitutional delay of puberty, idiopathic hypogonadotropic hypogonadism, and functional hypogonadotropic hypogonadism share several pathophysiological similarities: male predominance, familial predisposition, and disordered gonadotropin secretion.^{10,11} In patients with these disorders, the response to appropriate GnRH stimuli is the restoration of secretion of luteinizing hormone and follicle-stimulating hormone, which points to the primacy of the hypothalamic disorder in the pathogenesis of the disorders. Some patients with idiopathic hypogonadotropic hypogonadism, including those with known mutations of genes associated with this disorder, may undergo delayed activation of the hypothalamic–pituitary–testicular axis. This finding raises the possibility that idiopathic hypogonadotropic hypogonadism, constitutional delay, and functional hypogonadotropic hypogonadism — all characterized by disordered timing or regulation of the GnRH pulse generator — may result from analogous or overlapping pathophysiological mechanisms.

Raivio et al. speculate that exposure to androgen may have contributed to the maturation

of the GnRH neuronal network in men with idiopathic hypogonadotropic hypogonadism who had sustained reversal of the condition. Though plausible, this hypothesis does not explain clinical observations that some women with idiopathic hypogonadotropic hypogonadism may also undergo delayed activation of the GnRH pulse generator. Sex-specific exposure to sex steroids may facilitate neurogenesis and maturation of the neural network involved in pulsatile GnRH secretion. The developmental-clock genes that regulate the timing and maturation of the GnRH neuronal network receive important permissive signals from molecules indicative of somatic growth and nutritional status, and through environmental cues such as the photoperiod.¹² The multifaceted interactions of sex steroids, the GnRH neuronal network, and the developmental-clock genes are important players in an orchestra that directs the remodeling of the neural circuits that regulate the GnRH secretory network as well as sex-specific sexual behaviors.¹³ The identity of the developmental-clock genes and the mechanisms that integrate sensory input from environmental and social cues with energy balance, skeletal growth, and sex-steroid feedback remain poorly understood. It is likely that aberrations in the expression of developmental-clock genes and other integrating mechanisms will emerge as contributors to disorders of pubertal timing.

The primary disorders of gonadotropin secretion or action, such as those resulting from mutations in gonadotropin genes, can also partially or completely abrogate sexual development.^{4,14} Inactivating *LHB* mutations in men have been known to be associated with a lack of pubertal development as well as infertility.^{4,14,15} Lofrano-Porto et al. describe a woman with a newly discovered homozygous mutation in a 5' splice-donor site in the noncoding region of *LHB* that is associated with impaired luteinizing hormone secretion, normal pubertal development, secondary amenorrhea, and infertility. The splice-site mutation disrupts the splicing of intron 2, resulting in a mutant messenger-RNA transcript that contains an extra 236 nucleotides in homozygotes. Although luteinizing hormone secretion could not be detected by two separate immunoassays, whether the mutant *LHB* protein was translated but was unable to associate with the alpha subunit, was translated but rapidly degraded, or was not translated at all is uncertain. The phenotypic

features of the *LHB* mutation in the two affected men in this same report — failure of pubertal development, androgen deficiency due to a lack of maturation of Leydig's cells, and infertility due to spermatogenic arrest — are similar to those reported in men with other *LHB* mutations^{14,15} and in luteinizing-hormone beta-knockout mice.¹⁶ The new insight gained from the report by Lofrano-Porto et al. is that normal pubertal maturation in women, including breast development and menarche (which indicate estrogen production sufficient for breast development and at least some tropic action on the endometrium), can occur in a state of luteinizing hormone deficiency, although normal luteinizing hormone secretion is obligatory for ovulation. Thus, this rare experiment of nature supports the view that luteinizing hormone is essential for the normal maturation of Leydig's cells and steroidogenesis in men and that its primary role in women is to induce ovulation.

What are the implications of these findings? A vast majority of patients with constitutional delay or functional hypogonadotropic hypogonadism, and some men with idiopathic hypogonadotropic hypogonadism, are expected to undergo spontaneous puberty eventually; this statistic should prove very reassuring to patients and their parents. Even patients who have received a diagnosis of idiopathic hypogonadotropic hypogonadism because spontaneous puberty has failed to occur by 18 years of age and who are receiving sex-steroid-replacement therapy should periodically undergo a brief discontinuation of hormonal therapy to assess whether their endogenous gonadotropin secretion has normalized.

Constitutional delay, functional hypogonadotropic hypogonadism, and idiopathic hypogonadotropic hypogonadism may be a related group of disorders characterized by delayed activation of the GnRH pulse generator. Insights into the mechanisms by which delayed maturation of the GnRH secretory network is eventually reversed, resulting in the normal secretion of gonadotropin and testosterone in patients with these disorders, should help unravel the mysteries of the pubertal clock.

Dr. Bhasin reports receiving lecture fees from Indevus and grant support from Solvay Pharmaceuticals. No other potential conflict of interest relevant to this article was reported.

From the Section of Endocrinology, Diabetes, and Nutrition at Boston Medical Center and the Boston University School of Medicine — both in Boston.

1. Achermann JC, Weiss J, Lee EJ, Jameson JL. Inherited disorders of the gonadotropin hormones. *Mol Cell Endocrinol* 2001;179:89-96.
2. Seminara SB, Crowley WF Jr. The importance of genetic defects in humans in elucidating the complexities of the hypothalamic-pituitary-gonadal axis. *Endocrinology* 2001;142:2173-7.
3. Seminara SB. The first kiss — a crucial role for kisspeptin-1 and its receptor, G-protein-coupled receptor 54, in puberty and reproduction. *Nat Clin Pract Endocrinol Metab* 2006;2:328-34.
4. Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr Rev* 2000;21:551-83.
5. Raivio T, Falardeau J, Dwyer A, et al. Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med* 2007;357:863-73.
6. Lofrano-Porto A, Barra GB, Giacomini LA, et al. Luteinizing hormone beta mutation and hypogonadism in men and women. *N Engl J Med* 2007;357:897-904.
7. Quinton R, Cheow HK, Tymms DJ, Bouloux PM, Wu FC, Jacobs HS. Kallmann's syndrome: is it always for life? *Clin Endocrinol (Oxf)* 1999;50:481-5.
8. Bauman A. Markedly delayed puberty or Kallmann's syndrome variant. *J Androl* 1986;7:224-7.
9. Rosenfield RL. Diagnosis and management of delayed puberty. *J Clin Endocrinol Metab* 1990;70:559-62.
10. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab* 2002;87:1613-20.
11. Sedlmeyer IL, Hirschhorn JN, Palmert MR. Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. *J Clin Endocrinol Metab* 2002;87:5581-6.
12. Sisk CL, Foster DL. The neural basis of puberty and adolescence. *Nat Neurosci* 2004;7:1040-7.
13. Romeo RD, Sisk CL. Pubertal and seasonal plasticity in the amygdala. *Brain Res* 2001;889:71-7.
14. Weiss J, Axelrod L, Whitcomb RW, Harris PE, Crowley WF, Jameson JL. Hypogonadism caused by a single amino acid substitution in the β subunit of luteinizing hormone. *N Engl J Med* 1992;326:179-83.
15. Valdes-Socin H, Salvi R, Daly AF, et al. Hypogonadism in a patient with a mutation in the luteinizing hormone beta-subunit gene. *N Engl J Med* 2004;351:2619-25.
16. Ma X, Dong Y, Matzuk MM, Kumar TR. Targeted disruption of luteinizing hormone beta-subunit leads to hypogonadism, defects in gonadal steroidogenesis, and infertility. *Proc Natl Acad Sci U S A* 2004;101:17294-9.

Copyright © 2007 Massachusetts Medical Society.

EARLY JOB ALERT SERVICE AVAILABLE AT THE NEJM CAREERCENTER

Register to receive weekly e-mail messages with the latest job openings that match your specialty, as well as preferred geographic region, practice setting, call schedule, and more. Visit the NEJM CareerCenter at www.nejmjobs.org for more information.