Gonadotropic Axis Deficiency: A Neurodevelopmental Disorder

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Abstract The onset of puberty remains a mysterious mechanism despite several recent major breakthroughs in the field of neuroendocrinology. Novel neuropeptides, along with new regulation networks of gene expression, have been described; however, the intimate molecular mechanisms defining the age of puberty onset have yet to be characterized. This article proposes to broaden the reflection surrounding the mechanisms that determine the onset of puberty as a developmental process, similar to those described for other neuronal networks. This new and original approach will bring further insight into the intricate interactions between the brain and the gonads that drive and maintain normal reproductive function.

Puberty: A Neurodevelopmental Process That Starts Earlier Than Usually Thought

The thelarche is defined by the appearance of breasts in girls. It results from an increase in estrogen secretion by the ovaries and corresponds to the stage of the Tanner classification defined by the somatic changes occurring up to the end of puberty. Tanner stage 5 corresponds to a fully developed, mature individual with a functional reproductive activity. Although it is usually considered to be the first marker of puberty, Tanner stage 2 actually reflects a re-activation of the gonadotropic axis that had already started several months earlier. Indeed, biological puberty starts before clinical puberty through nocturnal elevation of plasma luteinizing hormone (LH), without changes during the day. Moreover, pituitary response to GnRH injection appears concomitantly to thelarche. In other words, the pituitary activation started several months before, probably by the nocturnal activation of the gonadotropic axis. The molecular mechanism of this circadian activation of the gonadotropic axis at the end of childhood is unknown. Further, from

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stages 2 to 5, plasma LH undergoes important variations as diurnal LH reaches the nocturnal levels and the circadian cycle completely disappears. In the meantime, the negative feedback by steroid hormones in both sexes as well as the positive feedback by estrogen in females only become functional on the hypothalamo-pituitary unit. From a biological point of view, puberty therefore corresponds to the development of a brain-gonads homeostasis that leads to normal reproductive function. This is unique to the gonadotropic axis.

The recent discovery of kisspeptins and their ability to tightly regulate GnRH neurons has led to a better understanding of the interplay between gonads and the brain, especially in regard to neurons and other hypothalamic cells that are involved in the regulation of GnRH secretion and associated disorders (de Roux et al. 2003; Pinilla et al. 2012). Known as the GnRH network, it is composed of neurosecretory neurons, glial cells, such as astrocytes, and also glia-like cells called tanycytes (Prevot et al. 2010). The main function of this network is to regulate GnRH secretion. For methodological and conceptual reasons, it is difficult to study and conceive of a constantly evolving neuronal network; hence the vast majority of original knowledge on the GnRH network has come from adult animals. The roles of kisspeptin, dynorphin, and/or neurokinin B have been described in detail, from their regulation by sexual hormones to the involvement of glial cells in this network (Lomniczi et al. 2013b; Tena-Sempere 2012). These results were then transposed to pre-pubertal animals and helped define the link between the increase of *KiSS1* expression in the hypothalamus and the onset of puberty (Terasawa et al. 2013).

These novel findings show that pubertal onset is the consequence of a long neurodevelopmental process that starts during fetal life, just like most neuronal networks do. The neurodevelopment of the gonadotropic axis is, however, unique for several reasons. GnRH neurons emerge from the olfactory placode and migrate to the hypothalamus during early pre-natal development. Such migration toward the brain is very uncommon in animal biology and has largely been studied in humans affected with Kallman syndrome and in rodents (Mitchell et al. 2011; Wierman et al. 2011). In primates, gonadotrope cells are functional and can response to GnRH stimulation at midgestation. The hypothalamo-pituitary control of the gonadotropic axis must be functional during fetal life to have complete development of gonads in both sexes and sexual organs in males. However, this control is a complex phenomenon. Indeed, at mid-gestation, blood levels of LH and follicle stimulating hormone (FSH) are elevated and close to those observed in adults, whereas at the end of gestation, LH and FSH blood levels are close to null (Guimiot et al. 2012). Such negative regulation partly depends on kisspeptins expressed in the infundibulum (Guimiot et al. 2012). It is important to note that this timing is specific to the gonadotropic axis since other endocrine axes tend to be activated at the end of gestation. The second part of gestation is therefore crucial to producing normal reproductive function in adults.

Post-natal development of the gonadotropic axis is comprised of several stages that are clinically well defined. Mini-puberty starts a few weeks after birth and is followed by a quiescent phase that goes on during childhood until puberty at the average age of 10. The mechanisms underlying this sequential activation-inactivation surrounding mini-puberty are poorly understood, the majority of

studies having essentially focused on the reactivation at puberty. In rodents, mini-puberty is not well characterized. It may correspond to the FSH surge observed in mice at post-natal day (PND12), knowing that puberty usually starts around PND30. This rather late surge, when compared to humans, may be explained by the fact that the first week of rodent life corresponds to the third semester of human in utero development. It was recently shown that the quiescent phase partly depends on a reduced kisspeptin tonus in non-human primates (Ramaswamy et al. 2013). The timing of this central activation of the gonadotropic axis in the first months of life is comparable to other neurodevelopmental processes.

In recent years, studies on puberty onset have brought to light that puberty is associated with a vast genes and transcription factors network whose hypothalamic expression either increases or decreases before pubertal onset (Lomniczi et al. 2013b). Indeed, this complex network notably depends on epigenetic regulations that modulate kisspeptin expression (Lomniczi et al. 2013a). These studies have been of tremendous importance to understanding puberty onset proceedings but have failed to bring answers to the question of pubertal timing. In other words, the molecular events leading to variations of hypothalamic genes expression through time remain unknown.

Neurodevelopment is not limited to the embryonic development of neurons. It also corresponds to the development of synaptic connections or synaptogenesis, which starts during fetal life and goes on throughout adulthood. After birth, neurons are myelinated, further modifying neuronal network activity. Synaptogenesis is composed of well-timed stages, and adolescence is a very important period of synaptic remodeling during which synaptic contacts between neurons get simplified to maximize efficacy. It is interesting to note that puberty happens concomitantly with such complex and intense regulation of synaptic activity in the brain (Sisk and Foster 2004). The link between these two events has essentially been studied through the effect of sexual hormones on brain organization (Ahmed et al. 2008). Nonetheless, it appears that the plasticity of cortical neurons during puberty only partially depends on sexual hormones. These results suggest that the GnRH network is organized and timely determined by similar processes to those involved in the normal plasticity of cortical neurons. This maturation is initially independent of sexual hormones, then progressively shifts under the control of estradiol and testosterone during puberty. Inhibitory input from GABA and excitatory input from glutamate on GnRH and Kisspeptin neurons during the early phase of puberty have hence been demonstrated in rodents. These inputs radically change during the childhood-to-puberty transition. We assume that this change is the consequence of the neurodevelopmental process that probably began during the second half of gestation and just after puberty.

The characterization of the neurodevelopmental mechanisms shared by both hypothalamic and cortical neurons will have a double impact. Firstly, it should provide further understanding of pubertal onset and its associated diseases. Secondly, it should provide new hypotheses to explain neurodevelopmental disorders in children.

Congenital Gonadotropic Deficiency: A Neurodevelopmental Anomaly of the Gonadotropic Axis

We initially studied isolated gonadotropic deficiency, which led us to characterize mutations in *GnRHR* and *KiSS1R* (de Roux et al. 1999, 2003). Mutations in *KISS1* (Topaloglu et al. 2012), *GnRH1* (Bouligand et al. 2009), and also *TAC3* encoding neurokinin B, and *TACR3,* the receptor of neurokinin B (Topaloglu et al. 2009) have also been described in isolated hypogonadotropic hypogonadism. The phenotype observed in these patients has confirmed that the central control of the gonadotropic axis during fetal life is required for normal development of reproductive function. Investigation of the neuroendocrine functions of Kisspeptin and Neurokinin B has revealed their role in the secretion of GnRH. In addition, several genetic defects have been described in Kallmann syndrome (for review see Pitteloud et al. 2010). All of these genes encode proteins required for the development of the olfactory bulbs (Pitteloud et al. 2010). The gonadotropic deficiency observed in these patients is mainly the consequence of the agenesis of the olfactory tracts, although a direct action of these proteins on the migration and maturation of GnRH neurons has also been proposed. We now have evidence that isolated gonadotropic deficiency is not only an endocrine disorder but is also a neurodevelopmental disorder due to a defect in the migration of GnRH neurons or the neuroendocrine control of GnRH neurons.

We have thus suspected that studying complex diseases comprising both neurological disorders and a delayed puberty due to a congenital gonadotropic deficiency should be very informative to delineating the mechanism of the initiation of puberty and related disorders. Indeed, we believe both conditions originate from the same neurodevelopmental anomaly. The literature shows that gonadotropic deficiency has been described in many neurological syndromes, some of which are extremely rare (Alazami et al. 2008; Bem et al. 2011; Bernard et al. 2011; Margolin et al. 2013; Nousbeck et al. 2008; Synofzik et al. 2014; Tetreault et al. 2011). Apart from Prader-Willi and CHARGE syndromes, which are well known by pediatric endocrinologists, most of these syndromes are only followed in pediatric neurology departments. Indeed, hypogonadotropic hypogonadism is rarely diagnosed at birth in these children.

The neurological disease can be neurodegenerative, hence progressive. Signs of dementia have been observed in some adults (Margolin et al. 2013), and 4H syndrome, which associated a leucodystrophia with hypodontia and hypogonadotropic hypogonadism, has been described (Bernard et al. 2011; Tetreault et al. 2011). Mental retardation is frequently observed, as well as cerebellar or proprioceptive ataxia. Some signs are specific to one of these syndromes, such as a microphtalmia associated with a cataract in Warburg microsyndrome (Handley et al. 2013). The endocrine phenotype has been more or less described, with in utero or post-natal growth retardation. In most cases, the endocrine deficit is limited to the gonadotropic axis and is of hypothalamic origin.

Several genes have been described via genetic linkage or exome analysis. These genes encode proteins with various and sometime unexpected functions. For instance, 4H syndrome is due to mutations in two type III RNA polymerase sub-units encoding genes, *POLR3A* and *POLR3B* (Bernard et al. 2011; Tetreault et al. 2011). This is a surprising result, since type III RNA polymerase is ubiquitous. Its main function is to transcribe transfer RNA genes, which will further participate in protein translation. Interestingly, other leucodystrophias involving a defective protein synthesis have been described, but no gonadotropic deficiency was observed. This indicates that the gonadotropic deficiency in 4H syndrome does not depend on white matter disease but on POLR3A or POLR3B dysfunction. Mutations in other genes, such as *RNF216* and *OTUD4*, were shown to cause a complex phenotype associating a gonadotropic deficiency with dementia. Again, it seems that synaptic activity may be impaired, though RNF216 and ITUD4 have been shown to be involved in protein ubiquitination, a major mechanism of protein degradation (Margolin et al. 2013). Very recently, mutations in *PNPLA6* were identified in Boucher-Neuhauser and Gordon Holmes syndromes (Synofzik et al. 2014). PNPLA6 belongs to the family of proteins with a phospholipid esterase domain. It is interesting to note that *PNPLA6* mutations were characterized in different neurodegenerative diseases with ataxia, suggesting that Boucher-Neuhauser syndrome is a phenotypic variant of an important group of neurodegenerative diseases.

Warburg microsyndrome is caused by mutations in *RAB3GAP1*, *RAB3GAP2* and *RAB18* (Handley et al. 2013). RAB3GAP1 and RAB3GAP2 are proteins with a GTPase activity on RAB3 and RAB27. RAB18 is a small monomeric G protein. These results suggest that the gonadotropic deficiency may result from a defective neuronal function such as a neuropeptide or neurosecretion defect.

Syndromic gonadotropic deficiency has thus been described in many neurological pathologies, some of which are extremely rare. Human genetics have led to the identification of several genes but the mechanisms leading to the gonadotropic deficiency remain poorly understood. Nonetheless, hypotheses arising from these results must be validated in animal models by neuroendocrinology laboratory teams to delineate the exact function of these proteins in the central control of the gonadotropic axis. To go further in this direction, our group has decided to characterize the molecular mechanism involved in a phenotype from three siblings. This particular phenotype regroups an intra-uterine and post-natal growth retardation, an impaired glucose metabolism leading to hypoglycemic episodes during childhood and insulino-dependent diabetes during adolescence, a proprioceptive ataxia, mental retardation, central hypothyroidism and a gonadotropic deficiency without anosmia. Our results showed that the haploinsuffisiency of DMXl2 was the cause of this familial phenotype (Tata et al. 2014).

Conclusions and Perspectives

The development of the molecular genetics of puberty disorders over the last 15 years has been critical to delineate puberty onset mechanisms. The constant technical evolution of molecular biology and close collaboration with pediatric endocrinology and pediatric neurology hospital departments will provide opportunities to identify novel genes involved in hypogonadotropic hypogonadism. Initially focused on isolated gonadotropic deficiency, recent promising results have thus been obtained from the study of syndromic forms. This new gene inventory will be of utmost interest for screening familial and sporadic cases of IHH, thus providing better information for genetic counseling.

Initially classified as an endocrine defect, isolated gonadotropic deficiency must now be considered as a neurodevelopmental disorder due to a defect in neuron migration, a defect in the neuroendocrine control of the GnRH network and also a defect in synaptic communication between neurons. This new view of the etiopathogenicity of pubertal disorders also offers new perspectives on the concept of neurodegenerative diseases specific to the GnRH network. Moreover, beyond the fundamental contribution to the mechanisms of puberty onset, they represent an opportunity to study extremely rare phenotypes. Finally, these works will help to understand the development of the homeostasis of the brain-gonad axis during childhood and adolescence that will lead to normal fertility throughout adult life.

References

Ahmed EI, Zehr JL, Schulz KM, Lorenz BH, DonCarlos LL, Sisk CL (2008) Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. Nat Neurosci 11:995–997

Alazami AM, Al-Saif A, Al-Semari A, Bohlega S, Zlitni S, Alzahrani F, Bavi P, Kaya N, Colak D, Khalak H, Baltus A, Peterlin B, Danda S, Bhatia KP, Schneider SA, Sakati N, Walsh CA, Al-Mohanna F, Meyer B, Alkuraya FS (2008) Mutations in C2orf37, encoding a nucleolar protein, cause hypogonadism, alopecia, diabetes mellitus, mental retardation, and extrapyramidal syndrome. Am J Hum Genet 83:684–691

Bem D, Yoshimura S, Nunes-Bastos R, Bond FC, Kurian MA, Rahman F, Handley MT, Hadzhiev Y, Masood I, Straatman-Iwanowska AA, Cullinane AR, McNeill A, Pasha SS, Kirby GA, Foster K, Ahmed Z, Morton JE, Williams D, Graham JM, Dobyns WB, Burglen L, Ainsworth JR, Gissen P, Müller F, Maher ER, Barr FA, Aligianis IA (2011) Loss-of-function mutations in RAB18 cause Warburg micro syndrome. Am J Hum Genet 88:499–507

Bernard G, Chouery E, Putorti ML, Tetreault M, Takanohashi A, Carosso G, Clement I, Boespflug-Tanguy O, Rodriguez D, Delague V, Abou Ghoch J, Jalkh N, Dorboz I, Fribourg S, Teichmann M, Megarbane A, Schiffmann R, Vanderver A, Brais B (2011) Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. Am J Hum Genet 89:415–423

Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J (2009) Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med 360:2742–2748

de Roux N, Young J, Misrahi M, Schaison G, Milgrom E (1999) Loss of function mutations of the GnRH receptor: a new cause of hypogonadotropic hypogonadism. J Pediatr Endocrinol Metab 12(Suppl 1):267–275

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 100:10972–10976

Guimiot F, Chevrier L, Dreux S, Chevenne D, Caraty A, Delezoide AL, de Roux N (2012) Negative fetal FSH/LH regulation in late pregnancy is associated with declined kisspeptin/KISS1R expression in the tuberal hypothalamus. J Clin Endocrinol Metab 97:E2221–E2229

Handley MT, Morris-Rosendahl DJ, Brown S, Macdonald F, Hardy C, Bem D, Carpanini SM, Borck G, Martorell L, Izzi C, Faravelli F, Accorsi P, Pinelli L, Basel-Vanagaite L, Peretz G, Abdel-Salam GM, Zaki MS, Jansen A, Mowat D, Glass I, Stewart H, Mancini G, Lederer D, Roscioli T, Giuliano F, Plomp AS, Rolfs A, Graham JM, Seemanova E, Poo P, García-Cazorla A, Edery P, Jackson IJ, Maher ER (2013) Mutation spectrum in RAB3GAP1, RAB3GAP2, and RAB18 and genotype-phenotype correlations in Warburg micro syndrome and Martsolf syndrome. Hum Mutat 34:686–696

Lomniczi A, Loche A, Castellano JM, Ronnekleiv OK, Bosch M, Kaidar G, Knoll JG, Wright H, Pfeifer GP, Ojeda SR (2013a) Epigenetic control of female puberty. Nat Neurosci 16:281–289

Lomniczi A, Wright H, Castellano JM, Sonmez K, Ojeda SR (2013b) A system biology approach to identify regulatory pathways underlying the neuroendocrine control of female puberty in rats and nonhuman primates. Horm Behav 64:175–186

Margolin DH, Kousi M, Chan YM, Lim ET, Schmahmann JD, Hadjivassiliou M, Hall JE, Adam I, Dwyer A, Plummer L, Aldrin SV, O'Rourke J, Kirby A, Lage K, Milunsky A, Milunsky JM, Chan J, Hedley-Whyte ET, Daly MJ, Katsanis N, Seminara SB (2013) Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. N Engl J Med 368:1992–2003

Mitchell AL, Dwyer A, Pitteloud N, Quinton R (2011) Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. Trends Endocrinol Metab 22:249–258

Nousbeck J, Spiegel R, Ishida-Yamamoto A, Indelman M, Shani-Adir A, Adir N, Lipkin E, Bercovici S, Geiger D, van Steensel MA, Steijlen PM, Bergman R, Bindereif A, Choder M, Shalev S, Sprecher E (2008) Alopecia, neurological defects, and endocrinopathy syndrome caused by decreased expression of RBM28, a nucleolar protein associated with ribosome biogenesis. Am J Hum Genet 82:1114–1121

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M (2012) Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 92:1235–1316

Pitteloud N, Durrani S, Raivio T, Sykiotis GP (2010) Complex genetics in idiopathic hypogonadotropic hypogonadism. Front Horm Res 39:142–153

Prevot V, Bellefontaine N, Baroncini M, Sharif A, Hanchate NK, Parkash J, Campagne C, de Seranno S (2010) Gonadotrophin-releasing hormone nerve terminals, tanycytes and neurohaemal junction remodelling in the adult median eminence: functional consequences for reproduction and dynamic role of vascular endothelial cells. J Neuroendocrinol 22:639–649

Ramaswamy S, Dwarki K, Ali B, Gibbs RB, Plant TM (2013) The decline in pulsatile GnRH release, as reflected by circulating LH concentrations, during the infant-juvenile transition in the agonadal male rhesus monkey (Macaca mulatta) is associated with a reduction in kisspeptin content of KNDy neurons of the arcuate nucleus in the hypothalamus. Endocrinology 154:1845–1853

Sisk CL, Foster DL (2004) The neural basis of puberty and adolescence. Nat Neurosci 7:1040–1047

Synofzik M, Gonzalez MA, Lourenco CM, Coutelier M, Haack TB, Rebelo A, Hannequin D, Strom TM, Prokisch H, Kernstock C et al (2014) PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. Brain: J Neurol 137:69–77

Tâta B, Huijbregts L, Jacquier S, Csaba Z, Genin E, Meyer V, Leka S, Dupont J, Charles P, Chevenne D, Carel JC, Leger J, de Roux N (2014) Haploinsufficiency of Dmxl2, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse. PloS Biol 12(9):e1001952

Tena-Sempere M (2012) Deciphering puberty: novel partners, novel mechanisms. Eur J Endocrinol 167:733–747

Terasawa E, Guerriero KA, Plant TM (2013) Kisspeptin and puberty in mammals. Adv Exp Med Biol 784:253–273

Tetreault M, Choquet K, Orcesi S, Tonduti D, Balottin U, Teichmann M, Fribourg S, Schiffmann R, Brais B, Vanderver A, Bernard G (2011) Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. Am J Hum Genet 89:652–655

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK (2009) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 41:354–358

Topaloglu AK, Tello JA, Kota LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B (2012) Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med 366:629–635

Wierman ME, Kiseljak-Vassiliades K, Tobet S (2011) Gonadotropin-releasing hormone (GnRH) neuron migration: initiation, maintenance and cessation as critical steps to ensure normal reproductive function. Front Neuroendocrinol 32:43–52