



Review

Isolated GnRH deficiency: A disease model serving as a unique prism into the systems biology of the GnRH neuronal network

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Contents

1. Normal development of the reproductive axis activity in humans	4
2. Isolated GnRH deficiency in humans	5
2.1. Phenotypic features.	5
2.1.1. Olfactory function.	6
2.1.2. The clinical spectrum of reproductive defects in humans with isolated GnRH deficiency	6
2.1.3. Time of onset of reproductive deficit and duration of reproductive failure	6
2.1.4. Patterns of GnRH secretory defects.	7
2.1.5. Non-reproductive phenotypes	8
3. An evolving understanding of the genetic architecture of GnRH deficiency	8
3.1. Only half of the genes causing isolated GnRH deficiency have been identified	8
3.2. Monogenic vs. oligogenic genetic architecture to human GnRH deficiency	9
3.3. Epigenetic interactions	10
4. Concluding remarks	10
References	10

1. Normal development of the reproductive axis activity in humans

Developmentally, the fetal GnRH pulse generator and its activation of the downstream gonadotrope and gonadal axis in humans is fully operational in both sexes by the end of the 1st trimester of

gestation (Grumbach, 1994). Subsequently, serum LH and FSH levels are suppressed and at the time of delivery are undetectable levels in umbilical cord blood, presumably secondary to the inhibitory effects of the pharmacological levels of placentally-derived sex steroids (Grumbach, 1990; Massa et al., 1992) and possibly the high levels of circulating kisspeptin (Seminara et al., 2006; Ramaswamy et al., 2007). Within minutes of birth, male infants exhibit a brief surge of LH and testosterone that persists for up to 12 h (Corbier et al., 1990; de Zegher et al., 1992). In the subsequent 1–2 weeks of the neonatal period, the declining levels of placentally derived inhibitory factors then reveal a re-awakened secretion of

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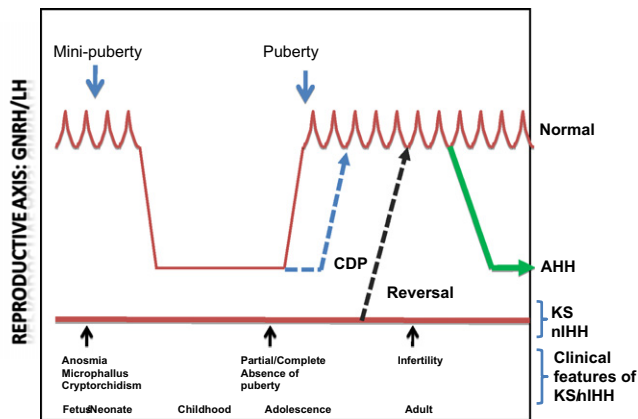


Fig. 1. Clinical presentations of isolated GnRH deficiency in humans. Pulsatile GnRH secretion is initiated during the late fetal/early neonatal period (“mini-puberty”), followed by quiescence during childhood and reawakening of the pulsatile secretion in adolescence. Presence of anosmia signals a developmental defect in GnRH neuronal migration while, microphallus or cryptorchidism signal fetal/neonatal lack of GnRH secretion. Constitutional delay of puberty (CDP) represents a late activation of the HPG axis while KS and nIHH represent partial or complete failure of pulsatile GnRH secretion. Recovery of pulsatile GnRH secretion in KS/nIHH subjects in adulthood is termed “Reversal”, while, AHH (adult-onset hypogonadotropic hypogonadism) refers to the onset of isolated GnRH deficiency during adulthood following a normal mini-puberty and puberty.

the hypothalamic GnRH pulse generator that results in the characteristic burst of GnRH-induced activity of the reproductive axis that is a hallmark of this period.

This neonatal burst of secretory activity of the hypothalamic–pituitary–gonadal axis, termed the “mini-puberty of infancy” (Fig. 1), differs in its duration between the two sexes. In males, LH, FSH, and testosterone levels peak between 4 and 10 weeks following which GnRH quiescence eventually is complete by 6 months (Forest, 1990; Andersson et al., 1998). In contrast, female infants show longer persistence of their GnRH secretory activation of their reproductive axes’ activity for up to 3 years, with hormonal values being highly variable during this time and characterized by an FSH predominance in striking contrast to the male (Walshauer et al., 1981; Andersson et al., 1998). In both sexes, this neonatal window of reproductive activation is driven by GnRH secretion and appears to serve at least two critical developmental agendas. In males, the first is a rapid expansion of the Sertoli cell population (Sharpe et al., 2003a,b) with a subsequent increase in the germ cell number that provides the underpinnings for subsequent fertility during adulthood (Muller and Skakkebaek, 1984). The second is exposure of all sex steroid dependent target organs (especially the brain) to a key developmental period of adult levels of sex steroids. This exposure has important organizational consequences for both immediate and subsequent development such as phallic development and testicular descent. Consequently, microphallus or micropenis (infantile penile size <2.5 cm) and cryptorchidism represent two phenotypic features that are the biological sequelae in boys when this brief window of *in utero*/neonatal activation of the HPG axis is defective in cases of human Isolated GnRH Deficiency (Fig. 1) (see below). Following this flurry of neonatal activity in both sexes, this activation of the HPG axis that is driven by GnRH secretion, is mysteriously dampened by unknown developmental ‘brakes’ that usher in a prolonged period of reproductive quiescence lasting throughout childhood. During this period of reduced sex steroids characteristic of childhood, a very low amplitude secretion of GnRH, gonadotropins and sex steroids continues that results in this nadir of reproductive activity that permits linear growth of the skeletal system to occur in the absence of epiphyseal fusion (Conte et al., 1975; Wu et al., 1990, 1991).

The gradual reawakening of the GnRH pulse generator during adolescence, initially during sleep and then equally during day and night, then heralds the onset of puberty (Fig. 1). In boys, this onset of puberty is initially signaled externally by isolated testicular enlargement. This selective activation of the germ cell compartment of the testes presumably reflects a secondary wave of GnRH-induced FSH secretion that is largely unrestrained by gonadal secretion of Inhibin B and, in fact, initiates Inhibin B biosynthesis and secretion during this ‘feed forward’ phase of FSH’s driving of Inhibin B production from the Sertoli cells. These relatively modest increases in FSH that occur during early puberty induce a second wave of seminiferous tubular proliferation, maturation of germ cells, and eventually the appearance of sperm in the ejaculate which is termed spermarche (Nielsen et al., 1986a,b). This period of active gametogenesis in the seminiferous tubular component of the testes is driven by FSH secretion. It is followed by penile and pubic hair growth driven by GnRH-induced LH’s activation on the Leydig cells and consequent secretion of testosterone. Eventually, the levels of Inhibin B secretion rise sufficiently to provide important negative feedback upon FSH secretion.

In girls, the appearance of pubic hair (pubarche) is initially driven by adrenal androgen secretion (adrenarche) and represents the typical first external sign of sexual maturation. This pubarche is soon followed by breast budding (thelarche), a spurt in linear growth, and much later menarche (Marshall and Tanner, 1969). This developmental cascade of maturational events is again initiated at the hypothalamic level by GnRH secretion and marks the second important biological and behavioral reproductive transition in humans whose control mechanisms have eluded scientists. In fact, Science magazine viewed the control of this process as one of the “125 great unanswered biological mysteries” in the current century (Kennedy and Norman, 2005). Given the fundamental elusiveness of this genetic control, the study of the human disease model of isolated GnRH deficiency has served as an important key to unlocking the secrets of puberty.

2. Isolated GnRH deficiency in humans

The advent of specific urinary and then serum assays for gonadotropins permitted classification of hypogonadal patients for the first time (Klinefelter and Griswold, 1943). Whereas most hypogonadal patients exhibited elevated levels of gonadotropins (hypergonadotropic hypogonadism), a much rarer subset of hypogonadal patients’ had normal or low gonadotropin levels with otherwise normal anterior pituitary function and CNS anatomy. While this condition was initially referred to as isolated gonadotropin deficiency prior to the discovery of GnRH (Heller and Nelson, 1948), once this hypothalamic decapeptide became available for human investigation in 1971, increases in the serum levels of LH and FSH following administration of a single bolus of GnRH in these subjects suggested that the defect was hypothalamic (Naftolin et al., 1971; Hashimoto et al., 1972; Roth et al., 1972). Eventually, administration of a physiologic pattern and dosing of exogenous GnRH to these patients complete restored a physiologic pattern and levels of gonadotropins and sex steroids, thus unequivocally demonstrating the hypothalamic nature of their defect (Crowley and McArthur, 1980; Hoffman and Crowley, 1982). Therefore, isolated GnRH deficiency is a more appropriate term to refer to this phenotype, as this nomenclature reflects the true pathophysiological defect in the vast majority of these patients.

2.1. Phenotypic features

Isolated GnRH deficiency presents a significant clinical and investigative challenge as well as an opportunity for new discovery

because of its heterogeneous clinical presentations and complex genetics. In both sexes, a broad phenotypic spectrum of baseline clinical presentations is seen that varies along several lines including the: (1) presence or absence of associated loss of smell (anosmia); (2) degree, severity, and timing of their reproductive defects; (3) patterns of endogenous GnRH secretory defects; and (4) critical presence or absence of non-reproductive defects.

2.1.1. Olfactory function

Historically, two forms of isolated GnRH deficiency have been clinically discernible: Kallmann syndrome (KS) in which isolated GnRH deficiency is accompanied by anosmia (lack of sense of smell) and its normosmic variant, idiopathic hypogonadotropic hypogonadism (nIHH). This critical developmental nexus between olfaction and reproduction was first appreciated in 1856 when Maestre de San Juan first described absent olfactory structures during the autopsy of an anosmic hypogonadal male (Maestre de San Juan, 1856). His report was followed by several pathologic and clinical reports of subjects with defective olfactory structures/function and hypogonadism (Weidenreich, 1914; Altmann, 1930; Kallmann et al., 1944; de Morsier, 1954; Gauthier, 1960; De Morsier and Gauthier, 1963). In 1944, Franz Kallmann (Kallmann et al., 1944) provided a more elaborate description of this syndrome that initiated the clinical investigation into this phenotype. Kallmann presented three pedigrees with multiple family members suffering from hypogonadism and anosmia; the anosmic variant has been referred to as Kallmann syndrome (KS) ever since. In addition to hinting at a possible genetic etiology by its familial pattern of occurrence, this seminal report also described other hypogonadal individuals who exhibited a normal sense of smell and other congenital anomalies. Although a precise anatomic and functional relationship between the olfactory structures and GnRH neurons was initially unclear, subsequent evidence from rodents and the detailed study of two human fetuses with a *KAL1* gene deletion eventually confirmed the common extra-CNS embryonic origins of GnRH neurons and olfactory axons and documented their failure to migrate into the CNS when the olfactory systems fails to develop in subjects with KS (Bick et al., 1989; Schwanzel-Fukuda and Bick, 1989). Thus, from this seminal report, it became clear from the various clinical phenotypes that at least two different types of underlying defects could be anticipated. In the first, developmental defects in olfactory system that result in anosmia were associated with the GnRH deficiency. In the others, no such developmental or neuroanatomic abnormality was presumably present; however, some neuroendocrine defect in GnRH regulation could be anticipated.

The KS variant of this syndrome accounts for nearly 60% of most series of isolated GnRH deficiency in the human (Seminara et al., 1998; Quinton et al., 2001; Seminara and Crowley, 2002) and is now viewed as a medical disorder that results from a developmental failure of GnRH neuronal migration (cf. below). Subjects with a normal sense of smell (nIHH), on the other hand, presumably represent a pure neuroendocrine defect in either secretion or action of GnRH. However clear such a dichotomous clinical distinction may seem between these two phenotypic variants, it is now increasingly evident that KS, nIHH or isolated anosmia are all phenotypes that can occur within a given affected family and thus represent some degree of phenotypic heterogeneity occurring within families. Indeed, this phenomenon was evident in Kallmann's original report (Kallmann et al., 1944). Given that Mendelian inheritance was assumed in this familial disorder, this incomplete penetrance and variable expressivity of phenotypes within KS/nIHH pedigree has long puzzled geneticists and clinical investigators. Several pedigrees of GnRH deficiency have now uncovered the role of gene/gene interactions (oligogenicity – cf. below) in this condition and thus demonstrate that these phenotypic variants of

GnRH deficiency line a much broader and more complex spectrum of clinical defects in isolated GnRH deficiency rather than representing single unique phenotypes. Such genetic and clinical heterogeneity sets the stage for a modern discovery period using genetic tools, the Human Genome Project's reference materials, and genomic and bioinformatic tools. Together, these new techniques have now provided the next and most interesting chapters in this biologic mystery of the genetic control of GnRH in the human as reviewed in this Special Edition of MCE.

2.1.2. The clinical spectrum of reproductive defects in humans with isolated GnRH deficiency

Constitutional delay of puberty (CDP) is a common and psychologically challenging adolescent phenotype that represents the mildest of reproductive defects in GnRH neuronal maturation in which the onset of GnRH secretion and hence puberty is delayed albeit normal once it occurs. CDP is defined statistically as the failure to initiate puberty beyond 2SD from the mean for a given population. CDP subjects, typically 5:1 male to female ratio, eventually enter puberty spontaneously albeit late but characteristically remain reproductively normal thereafter (Fig. 1). In contrast, subjects with the more complete form of isolated GnRH deficiency fail to either initiate or complete puberty resulting in either KS or nIHH and these subjects display varying degrees of pubertal impairment. For example, in some males with KS and nIHH, disruption of GnRH neuronal function and presumably pulsatile GnRH secretion occurred during the window of late fetal/early infancy (mini-puberty) leaving the phenotypic sequelae of micropenis and/or cryptorchidism that persist into adulthood and witness this earlier deficit in GnRH secretion (Fig. 1). Such a picture is typical of subjects with Neurokinin B deficiency as outlined in Chapter 8 (Gianetti et al., 2010). This combination of micropenis and cryptorchidism thus offers a potential clinical investigative opportunity to detect the consequences of impaired mini-puberty of the neonatal period thereby enabling the identification and diagnosis of those patients with GnRH deficiency at the earliest possible period in their post-natal life. If this window of opportunity to make the diagnosis is missed early in life and no other non-reproductive feature (e.g., anosmia, cleft lip/palate, renal agenesis, mental retardation, hearing defect, etc.) is present, the identification of subjects with isolated GnRH deficiency must await their hallmark failure to undergo a spontaneous and normally timed puberty when they ultimately will declare themselves to their physicians.

Complete absence of puberty is the next developmental window when GnRH deficiency becomes apparent clinically (Fig. 1). These GnRH deficient subjects present with a lack of any pubertal signs and symptoms of sexual maturation. They display frank hypogonadism with low gonadotropins, normal neuroanatomic studies, an isolated defect in gonadotropin secretion with other endocrine functions being intact, and infertility in both sexes. In a small percentage of such men, a partial GnRH deficient phenotype can be observed as the "fertile eunuch" variant of this syndrome. In these subjects cases, testicular steroidogenesis is nearly completely absent as evidenced by their frankly hypogonadal testosterone levels yet spermatogenesis is somewhat paradoxically preserved (McCullagh et al., 1953; Faiman et al., 1968; Makler et al., 1977). Similar to their male counterpart, females with IHH subjects can vary from complete absence of puberty with infertility to partial defects often when isolated thelarche or menarche may occur but fail to progress (Shaw et al., 2011).

2.1.3. Time of onset of reproductive deficit and duration of reproductive failure

2.1.3.1. Adult onset isolated GnRH deficiency.

The most severe form of GnRH deficiency derives from either the absence of GnRH secretion during either the "mini-puberty" of the neonatal period (with

microphallus and cryptorchidism) or more commonly, simply with absence of puberty. However, a more delayed presentation is now well-recognized in men who have undergone an otherwise completely normal sexual development at puberty but in whom hypothalamic secretion of GnRH subsequently ceased during adulthood (Nachtigall et al., 1997). This variant of isolated GnRH deficiency is termed adult-onset idiopathic hypogonadotropic hypogonadism whose responsiveness to exogenous GnRH administration in 9/10 cases demonstrates the hypothalamic nature of their defect (AHH) (Fig. 1). Long term follow of this syndrome has indicated these men fail to ever recover suggesting a permanent defect in GnRH secretion (Dwyer et al., 2010) as opposed to what happens in women with hypothalamic amenorrhea.

2.1.3.2. Hypothalamic amenorrhea. A milder adult onset but reversible form of functional GnRH deficiency occurs in otherwise normal adult females and was first referred to as 'hypothalamic amenorrhoea' (HA) by Albright and his colleagues (Moldawer et al., 1958). Hypothalamic amenorrhea is the most common reproductive disorder occurring in 2–3% of the normal female population (Pettersson et al., 1973). Whereas most of these subjects had previously experienced a normal puberty and sexual development, they subsequently develop transient hypogonadotropic hypogonadism due to defects in endogenous GnRH secretion that appears to be precipitated by excessive stress, under-nutrition, and/or over-exercise (Santoro et al., 1986). A key to this diagnosis and a hallmark of this syndrome attesting to the functional nature of this condition is that the HPG axis generally recovers in most upon removal of the precipitant. Whereas earlier thinking about women with hypothalamic amenorrhea stressed the role of these environmental factors such as exercise and nutrition, recent studies from

our group have indicated that even this mildest form of functional GnRH deficiency has a strongly underlying genetic basis (Caronia et al., 2011).

2.1.3.3. Reversible GnRH deficiency. Finally, although isolated GnRH deficiency was initially thought to be a life-long condition, spontaneous reversals of well-established GnRH deficiency have now been demonstrated to occur in approximately 10–15% of men with GnRH deficiency following therapy with a variety of treatments having in common elevation of sex steroids to pubertal levels (Raivio et al., 2007) (Fig. 1). This unique observation hints at a previously unrecognized plasticity of the GnRH neuronal network that can override developmental and neuroendocrine defects in GnRH neuronal ontogeny. It also implies that the GnRH neuronal network is in place anatomically so that it can subsequently secrete GnRH in a pulsatile fashion and awaken the pituitary–gonadal axis later in adulthood but that a failure of this mechanism occurs at the time of normal puberty. Since all of these patients received some degree of elevations of their gonadal sex steroids as part of their treatment, it is also suggested that exposure to sex steroid hormones is a key element to the milieu in which these 'reversals' occur.

2.1.4. Patterns of GnRH secretory defects

In keeping with the variable degree and timing of reproductive defects, the pattern of GnRH-induced gonadotropin secretion in subjects with isolated GnRH deficiency similarly displays a wide spectrum of neuroendocrine defects in both sexes (Santoro et al., 1986; Spratt et al., 1987; Shaw et al., 2011). A normal GnRH-induced LH secretion pattern in adult male is shown in Fig. 2A. Although the majority of subjects display an undetectable LH pulse profile (termed an apulsatile pattern, Fig. 2B), some GnRH

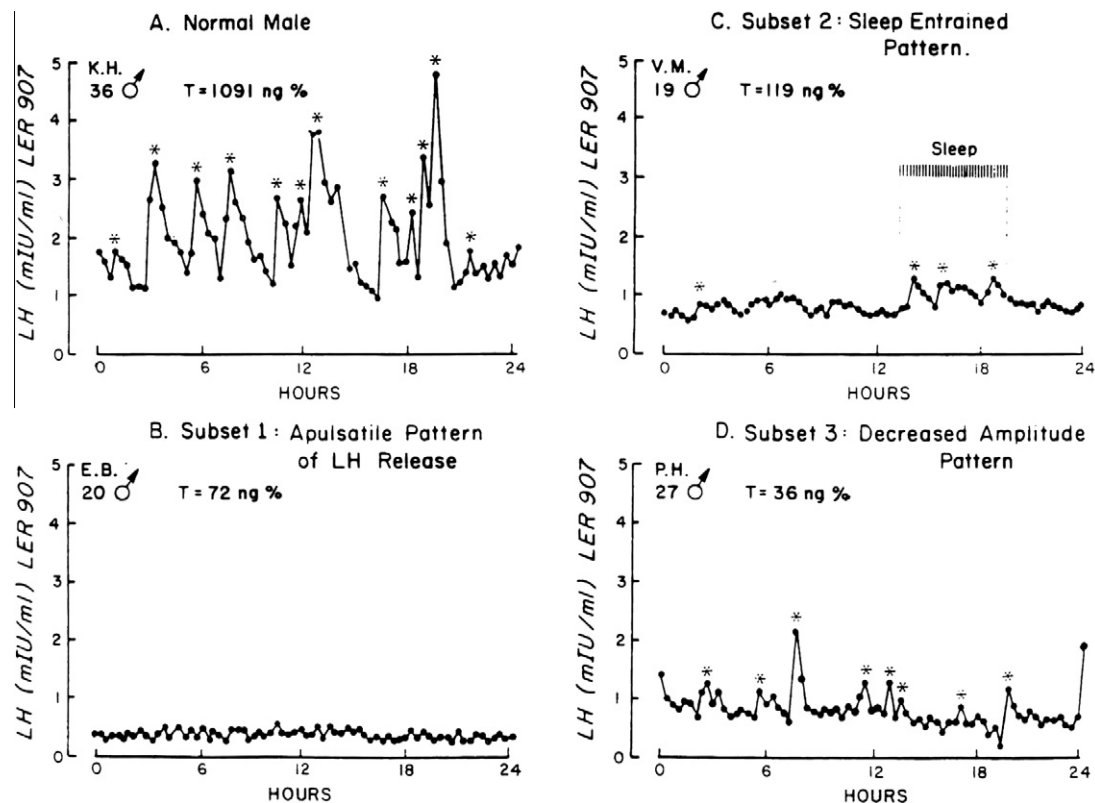


Fig. 2. GnRH secretory patterns in normal male and males with isolated GnRH deficiency. (A) Normal adult male pattern of LH secretion: High amplitude LH pulses at 2 h interval intervals with normal testosterone levels. (B) Apulsatile pattern of LH secretion in a GnRH deficient male subject with frank hypogonadal T levels. (C) Developmental arrest or sleep entrained (hatched bar) pattern of LH secretion in a GnRH deficient male. (D) Normal frequency but low amplitude pattern of LH secretion in a GnRH deficient male subject (Reprinted with permission from Santoro et al., *Endocr Rev.* 7(1):11–23).

deficiency subjects show preserved sleep-augmented LH pulse secretion such as is typically seen in mid-puberty (developmental arrest pattern, Fig. 2C) while yet another minority of patients demonstrate the presence of an enfeebled, low amplitude LH secretion occurring at a normal frequency (Fig. 2D). In females with HA, frequent sampling studies during the follicular phase show a similar spectrum of defects in GnRH-induced LH secretion (Santoro et al., 1986; Caronia et al., 2011).

2.1.5. Non-reproductive phenotypes

Several non-reproductive features are now well-recognized to accompany GnRH deficiency and often provide the phenotypic clues as to the underlying gene defect. Therefore, these phenotypic features should be sought and used to assist in targeting genetic testing more effectively (Genetic Counseling Chapter 7). These well-described associations include: cranio-facial defects (cleft lip/palate, high arched palate, coloboma, choanal atresia); renal agenesis, horseshoe kidney and GU duplications; skeletal defects – digital anomalies (short 4th metacarpals; campylodactyly, syndactyly, clinodactyly), scoliosis; sensorineural deafness, dental agenesis, oculomotor abnormalities, bimanual synkinesia and cerebellar ataxia. Going forward, detailed phenotyping of GnRH deficient subjects with regards to these associated features is imperative to allow targeted diagnostic genetic testing.

3. An evolving understanding of the genetic architecture of GnRH deficiency

With the mounting genetic evidence and identification of genetic mutations outlined in this Special Edition, several overview

features about the genetic architecture of human GnRH deficiency have become evident:

3.1. Only half of the genes causing isolated GnRH deficiency have been identified

Given the impact of genes causing GnRH deficiency on family size prior to any treatment, most cases of isolated GnRH deficiency occurs in the sporadic form (Seminara and Crowley, 2002). Even in familial cases, the pedigrees of this disorder are characteristically small, thus hindering the ability to apply traditional linkage analysis as would be possible in larger families. Despite these hurdles, in the last two decades, several independent groups have documented and mapped 14 genes that lead to human GnRH deficiency. This rapid progress has been possible in a large part by the use of targeted clinical investigation, the evolving power of human genetics, combined with the tools and information derived from mapping the human genome. However, striking allelic heterogeneity is evident in each of the discovered genes and almost all of the genetic variants uncovered to date have been private mutations without any obvious mutational hot spots. Moreover, these currently known genes account for only half of the genetic basis of this disorder with each gene contributing only a minor percentage (Fig. 3). The role of each gene in the GnRH neuronal ontogeny varies from purely neurodevelopmental genes that impair GnRH development and migration (*KAL1*, *NELF*) to purely neuroendocrine genes (*GNRH1*, *GNRHR*, *KISS1*, *KISS1R*, *TAC3*, *TACR3*) while certain genes are implicated in both development and neuroendocrine function (*FGF8*, *FGFR1*, *PROK2*, *PROKR2*) (Fig. 4).

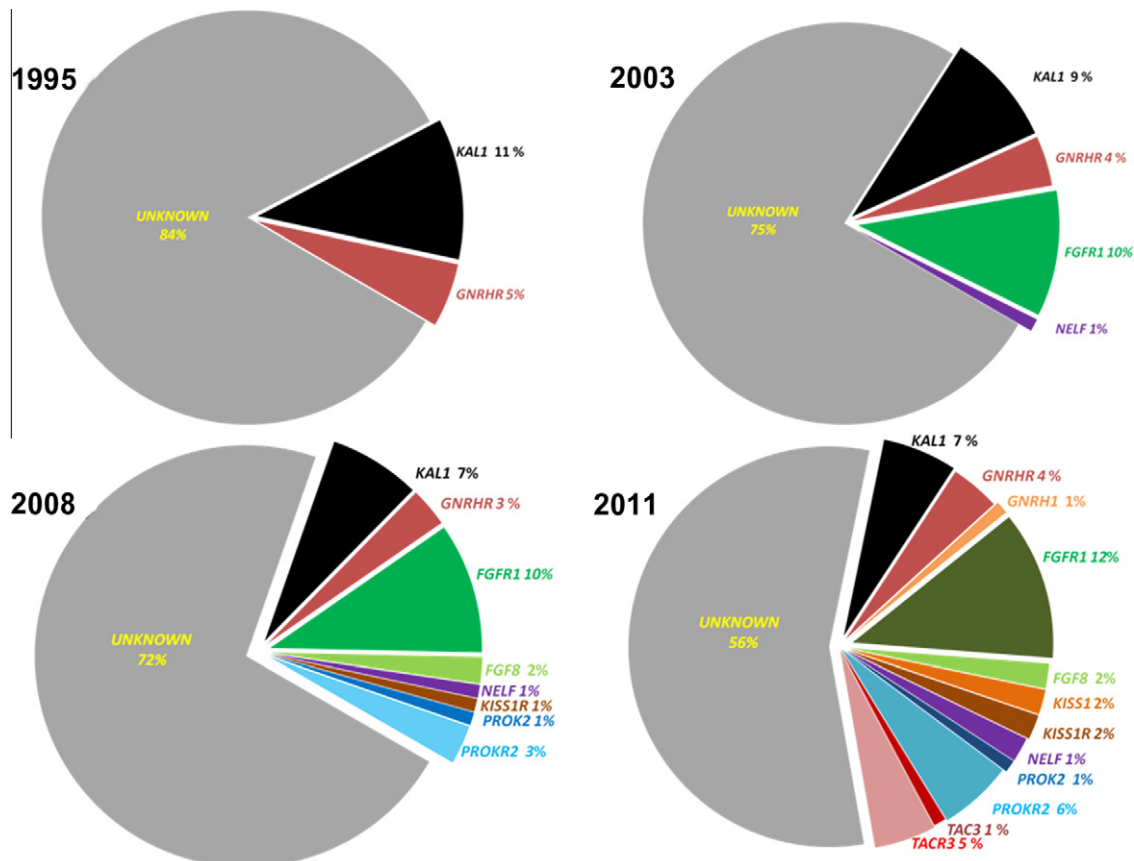


Fig. 3. Genetic causes of isolated GnRH deficiency: a historical perspective. Genetic etiology of isolated GnRH deficiency with relative percentage contribution from each identified gene to the heritability of the syndrome from 1995 to 2011. From only two genes that were known in 1995, the number of genes discovered in subjects with isolated GnRH deficiency has steadily increased through 1995–2011. In 2011, the genetic cause of a nearly half of the subjects is known while in the remaining half, the genes are yet to be identified (Data from the Harvard Reproductive Endocrine Sciences Center, Massachusetts General Hospital, Boston, MA).

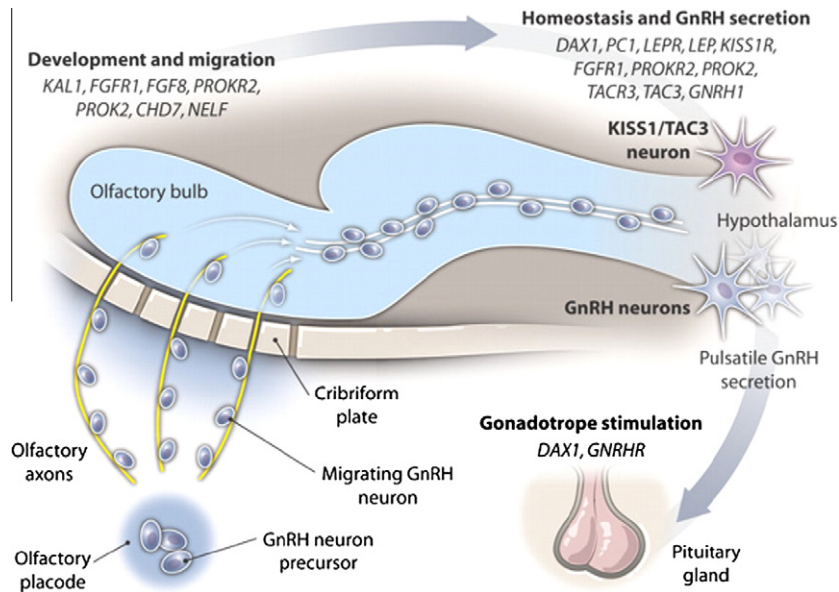


Fig. 4. GnRH neuronal ontogeny in humans. Specific gene mutations in patients with GnRH deficiency result in failure in GnRH development and migration (*KAL1*, Kallmann syndrome 1 sequence, MIM: 308700; *NELF*, nasal embryonic LHRH factor, MIM: 608137) while other gene mutations impair hypothalamic GnRH homeostasis and GnRH secretion (*DAX1*, dosage-sensitive sex reversal, adrenal hypoplasia congenital critical region on the X chromosome, gene 1, MIM: 300473; *PC1*, prohormone convertase 1, MIM: 162150; *LEPR*, leptin receptor, MIM: 601007; *LEP*, leptin, MIM: 164160; *KISS1R*, *KISS1* receptor, MIM: 604161; *TACR3*, tachykinin receptor 3, MIM: 162332; *TAC3*, tachykinin 3, MIM: 162330; *GNRH1*, gonadotropin-releasing hormone 1, MIM: 152760; *GNRHR*, GnRH receptor, MIM: 138850). Some gene mutations affect both migration/development as well as neuroendocrine secretion of GnRH (*FGFR1*, fibroblast growth factor receptor 1, MIM: 136350; *FGF8*, fibroblast growth factor 8, MIM: 600483; *PROKR2*, prokineticin receptor 2, MIM: 607123; *PROK2*, prokineticin 2, MIM: 607002; *CHD7*, chromodomain helicase DNA binding protein 7, MIM: 608765) (Reprinted with permission from Sykiotis GP et al., *Sci Transl Med.* 2010;2:32rv2).

Thus, the significant challenge that lies ahead is to utilize the evolving genetic techniques to identify the “missing heritability” in those familial cases of GnRH deficiency. In addition, it is likely that the genes yet to be discovered may be extremely rare variants; there may be several more of them; and thus each will account for only a minor percentage of the etiology of GnRH deficiency. Hence, large numbers of patients, extensive phenotypic databases, and collaborations across multiple groups with well-phenotyped cohorts of these rare patients will be the key to enhance gene discovery in the future. A more intriguing question is to whether the novel missing genes will map to the existing systems biology of the GnRH neurons or if they will unearth completely novel systems biology pathways. To the delight of basic and clinical investigators, the recent discovery of the novel kisspeptin pathway (de Roux et al., 2003; Seminara et al., 2003) has opened up an entirely new chapter in the study of the neurobiology of GnRH neuronal function and highlighted the critical role of the kisspeptin signaling system to control sexual maturation in all mammals studied to date (Seminara and Crowley, 2008; Oakley et al., 2009). It is tempting to speculate that similar novel insights may be expected from future investigation of this unique human phenotype.

3.2. Monogenic vs. oligogenic genetic architecture to human GnRH deficiency

The mode of inheritance in several GnRH deficient families with identified genetic variants poses an interesting genetic puzzle whose perspective has been dramatically changed by recent clinical investigations. For example, most pedigrees of male subjects harboring *KAL1* mutations display a fully penetrant expression of their reproductive and olfactory phenotypes (Hardelin et al., 1993; Izumi et al., 2001; Bhagavath et al., 2007; Versiani et al., 2007; Hardelin and Dode, 2008; Salenave et al., 2008) (see Chapter 2). Similarly, in subjects with homozygous mutations in presumed autosomal recessive genes (e.g., *GNRH1*, *GNRHR*, *TAC3*,

TAC3R, *PROK2*, *PROKR2*, *KISS1R*), segregation analysis generally reveals concordance of reproductive and non-reproductive phenotypes among mutation carriers (de Roux et al., 1999; Beranova et al., 2001; Dode et al., 2006; Seminara and Crowley, 2008; Bouligand et al., 2009; Chan et al., 2009; Gianetti et al., 2010; Martin et al., 2010; Sarfati et al., 2010). However, in pedigrees with heterozygous mutations in several of the known GnRH deficiency genes (e.g., *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, and *GNRHR*), multiple family members harboring identical mutant alleles display considerable phenotypic variability within a pedigree in terms of both their reproductive and non-reproductive features (Dode et al., 2006; Pitteloud et al., 2007b; Cole et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008; Martin et al., 2010; Sarfati et al., 2010). Similarly, the identical mutation in several different families can equally vary widely in their phenotype (Dode et al., 2006; Pitteloud et al., 2007b; Martin et al., 2010). Moreover, in some of these pedigrees, the mutation can occur in a phenotypically normal individual (Dode et al., 2006; Martin et al., 2010). Although these observations were initially puzzling, apparently violating the rules of strict Mendelian segregation analyses, the subsequent identification of digenic pedigrees where two individually rare pathogenic DNA sequence variants synergize or act in concert (digenicity or oligogenicity) to produce the phenotype being observed began to at least explain the observed variability encountered in many of these families (Pitteloud et al., 2007a). Though initially discovered in only two families (Pitteloud et al.), subsequent larger studies of nearly 400 GnRH deficient subjects have documented that digenicity occurs in nearly a quarter of all of those in which rare sequence variants could be identified (Sykiotis et al., 2010). This systematic evaluation of pathogenic rare variants in a large number of GnRH deficient patients has now shown that the prevalence of oligogenicity within human GnRH deficient subjects is at least 15% of patients with demonstrable coding sequence mutations (Sykiotis et al., 2010) (Fig. 5). Given that only 40–50% of the genetic causes are currently known (Fig. 3) and extrapolating this frequency of

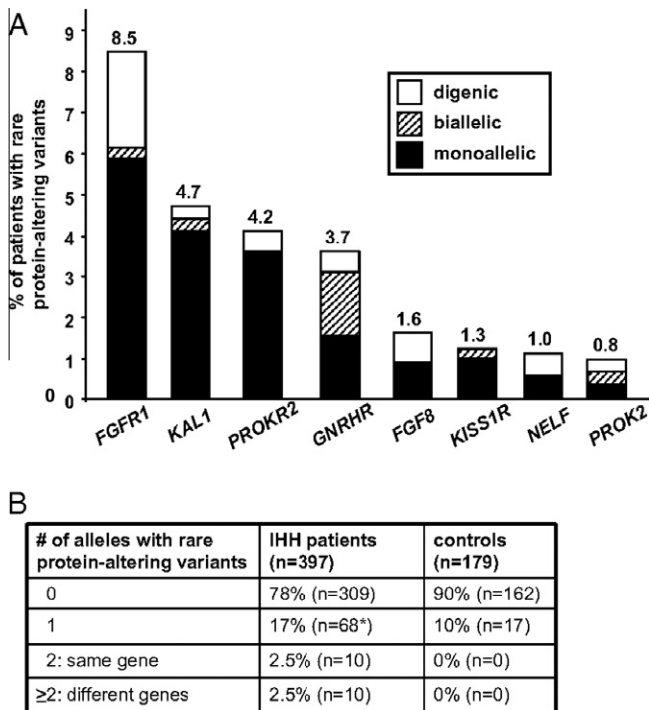


Fig. 5. Oligogenicity in isolated GnRH deficiency. (A) Frequency of monoallelic, biallelic, and digenic rare protein-altering variants in eight genes known to cause isolated GnRH deficiency. (B) Number of alleles with rare protein-altering variants in isolated GnRH deficient patients (IHH subjects) and healthy controls (Reprinted with permission from Sykiotis GP et al., Proc Natl Acad Sci USA. 2010; 107:15140-15144).

oligogenicity to unidentified genes, it is likely that GnRH deficiency in humans will ultimately prove to be an oligogenic human disorder rather than a purely monogenic condition as previously assumed. This oligogenic architecture is now known to occur in several other human disease models such as the ciliopathies (Badano et al., 2003). Thus, it is fairly likely that similar oligogenic mechanisms may partly explain the hitherto unexplained variable expressivity that has been puzzled clinicians in several monogenic Mendelian disorders.

3.3. Epigenetic interactions

Although the quest for the missing heritability in many genetic disorders has been focused on protein altering DNA sequence variants, it is increasingly clear that epigenetic changes may well contribute to the phenotypic heterogeneity in several human diseases. The notion that epigenetic interactions may play a role in the genetic architecture of GnRH deficiency comes from two observations. Despite a shared genetic background, monozygotic twins discordant for GnRH deficiency have been described (Hipkin et al., 1990; Seminara and Crowley, 2002). Additionally, siblings sharing a large deletion in *KAL1* gene exhibit considerable phenotypic heterogeneity (Massin et al., 2003). These observations strongly suggest the existences of potential modifying influences above and beyond mere sequence variations that somehow shape the eventual clinical phenotype of this condition. In addition, despite carrying clearly pathogenic mutations, several GnRH deficient individuals show reversal of their GnRH deficiency later in adulthood following exposure to sex steroids (Raivio et al., 2007). This recovery of GnRH neuronal function, seen in 10–15% of these patients, is highly suggestive of significant gene/environment/hormonal interactions (Raivio et al., 2007). In addition, it is highly likely that the GnRH neuronal network is not fixed in its capacity

during adulthood as evidenced by both the reversal phenotype and the occurrence of AHH in otherwise normal individuals (Nachtigall et al., 1997).

4. Concluding remarks

The scientific impetus for studying the molecular genetics of human GnRH deficiency is pinned on the hopes of using these unique insights to understand the biological basis of this disorder and to translate these insights into an improved understanding of the complex dynamics of common reproductive disorders as well as to design improved diagnostic and therapeutic strategies in these patients who have been such important partners in the discovery efforts of their condition. The physiologic and genetic studies in humans with isolated GnRH deficiency in the last two decades have just begun to chart the ontogenic determinants of the development and neuro-secretory functioning of GnRH neurons in a unique and powerful fashion. These studies have highlighted a key role of oligogenicity and gene/environment interactions, both of which are likely to underlie the phenotypic diversity in this disorder and guide our understanding in several other genetic diseases. The recent discovery that GnRH deficiency related genes also underlie the susceptibility to HA, the commonest reproductive disorder affecting 2–3% of otherwise normal women (Pettersson et al., 1973), provides an important proof of principle that detailed study of rare disorders such as GnRH deficiency can translate directly to common reproductive diseases.

Several challenges remain with a large number of genetic variants yet to be discovered, their expanding clinical diversity, the expensive phenotyping requirements of studying these patients, and the availability of new tools with which to probe this disorder. All of these opportunities and challenges strongly argue that this investigatory journey must continue and that collaborations and consortia will be the key to its future. The forthcoming years promise further novel insights with the advent of large-scale deep sequencing using next generation techniques. This opportunity begs an important question for the field. Are we ready to tackle the tsunami of the genetic variants that will emerge by the use of these advanced techniques? Can we collaborate effectively to do this as increasingly the ‘numbers game’ aspect of this will be important? Are we able to conduct the careful and comprehensive phenotyping of both reproductive and non-reproductive features of GnRH deficient subjects that will now be important than ever? Are we able to develop sufficiently robust functional assays to evaluate the role of these allelic variations singly and in combination with other mutations as only then can we begin to translate the newly acquired genomic information into the right clinical context?

There has never been a better time to assemble an international network of clinical investigators to collaborate and work in unison to help solve one of the 21st century’s unanswered puzzles: What triggers puberty?

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