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REVIEW

New understandings of the genetic basis of isolated idiopathic central hypogonadism

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Idiopathic hypogonadotropic hypogonadism is a rare disease that is characterized by delayed/absent puberty and/or infertility due to an insufficient stimulation of an otherwise normal pituitary–gonadal axis by gonadotrophin-releasing hormone (GnRH) action. Because reduced or normal luteinizing hormone (LH)/follicle-stimulating hormone (FSH) levels may be observed in the affected patients, the term idiopathic central hypogonadism (ICH) appears to be more appropriate. This disease should be distinguished from central hypogonadism that is combined with other pituitary deficiencies. Isolated ICH has a complex pathogenesis and is fivefold more prevalent in males. ICH frequently appears in a sporadic form, but several familial cases have also been reported. This finding, in conjunction with the description of numerous pathogenetic gene variants and the generation of several knockout models, supports the existence of a strong genetic component. ICH may be associated with several morphogenetic abnormalities, which include osmic defects that, with ICH, constitute the cardinal manifestations of Kallmann syndrome (KS). KS accounts for approximately 40% of the total ICH cases and has been generally considered to be a distinct subgroup. However, the description of several pedigrees, which include relatives who are affected either with isolated osmic defects, KS, or normo-osmic ICH (nICH), justifies the emerging idea that ICH is a complex genetic disease that is characterized by variable expressivity and penetrance. In this context, either multiple gene variants or environmental factors and epigenetic modifications may contribute to the variable disease manifestations. We review the genetic mechanisms that are presently known to be involved in ICH pathogenesis and provide a clinical overview of the 227 cases that have been collected by the collaborating centres of the Italian ICH Network.
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INTRODUCTION

The physiological function of the human hypothalamic–pituitary–gonadal (HPG) axis is based on the pulsatile release of the hypothalamic gonadotrophin-releasing hormone (GnRH), which is secreted into the hypophyseal portal circulation at the median eminence. The interaction of GnRH with its specific receptor (GnRH receptor (GnRHR)) on the pituitary gonadotropes stimulates the synthesis and secretion of the two pituitary gonadotrophins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH activate gonadal activity and gametogenesis in both sexes. GnRH-secreting neurons arise in the olfactory placode of the human embryo and then migrate to their final destination in the mediobasal hypothalamus to terminate their differentiation and initiate the secretion of GnRH.[1,2] This migration process is regulated by several factors, including the spatiotemporal expression patterns of axonal guidance cues, cell adhesion molecules, extracellular matrix proteins, and

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growth factors and neurotransmitters that determine the GnRH neuronal fate.[3] The correct development and coordinated function of the GnRH-secreting neurons and the gonadotropes is essential for the correct activation of the gonads during foetal life and the neonatal period (the so-called 'minipuberty'). After a dormant phase during infancy and childhood, HPG activity is resumed at the time of the puberty and throughout the adult reproductive age.

Central hypogonadism (CH) is either congenital or acquired, and it can be secondary to hypothalamic or pituitary lesions. CH may also be isolated or combined with other pituitary hormone defects.[4] Here, we will focus on the isolated forms of CH that cause variable defects of pubertal development and infertility; these forms are generally classified as idiopathic central hypogonadism (ICH). ICH is a disease with a complex and still largely unknown pathogenesis that is caused by an insufficient stimulation of an otherwise normal pituitary–gonadal axis by GnRH action. In fact, ICH is biochemically characterized by low levels of sex steroids (testosterone or estradiol) in the presence of low/normal levels of gonadotrophins (LH and FSH). In patients with normal levels of circulating gonadotrophins, LH and FSH are not secreted in a pulsatile manner and are therefore ineffective at the target level.[5] ICH is a rare disease; its incidence is 1 : 8000 males and 1 : 40 000 females. It can be associated with either a normal or defective sense of smell, which identifies normo-osmic ICH (nICH) or Kallmann syndrome (KS), respectively. Males frequently present with defective androgenisation and growth at a peripubertal age, but micropenis and cryptorchidism may already be evident in the neonatal period, which indicates a defective activation of HPG during prenatal development. Females generally present with primary amenorrhea and growth retardation. Additional neurological (e.g., anosmia) and non-neurological (e.g., midline or kidney defects) defects may frequently coexist, and they can be linked to specific modes of inheritance. Indeed, KS was originally thought to be caused by mutations in a specific X-chromosome gene, *KAL1*,[6–8] but this genetic defect was soon found to be present in only a minority of the patients. Nevertheless, the observation of familial cases with variable modes of ICH inheritance (X-linked or autosomal dominant or recessive) soon indicated that ICH retains a highly heterogeneous genetic component. The application of conventional linkage studies to investigate the genetic basis of ICH has proven to be difficult; most pedigrees tend to be small in size because the majority of patients remain infertile without treatment.[9] In the past decade, however, key contributions (reviewed in Ref. 5) from animal and cellular models, and genetic studies in a series of patients have provided evidence of new genetic determinants of ICH (either nICH or KS). These advances have played a significant role in the elucidation of the pathophysiology of ICH, and they have helped to discover the physiological complexities of the HPG axis.

GENETICS OF ICH

A multitude of candidate genes for ICH have been proposed, and a tentative schematic representation of the various mechanisms that can be disrupted by these genetic defects is reported in **Figure 1**. Some genes are required for the correct embryonic differentiation of the GnRH-secreting neurons, such as the receptor–ligand pair *FGFR1/FGF8* (*fibroblast growth factor receptor 1* and *fibroblast growth factor 8*), *NELF* (*nasal embryonic LH-releasing hormone Factor*) and *HS6ST1* (*heparan sulphate 6-O-sulphotransferase 1*). Other genes encode the signals that are essential for the correct migration of the GnRH neurons from their embryonic origin to the hypothalamus, such as *KAL1*, the ligand–receptor complex *PROK2/PROKR2* (*prokineticin 2* and its receptor) and *CHD7* (*chromodomain helicase DNA-binding protein 7*). Other genes encode the elements of upstream signals that contribute to the activation of the GnRH neuron, such as the two ligand–receptor complexes that are formed either by *TAC3/TACR3* (*tachykinin 3* and its receptor, which is also named *neurokinin B* (*NKB*), and *neurokinin 3 receptor* (*NK3*)) or *KISS1/KISS1R* (*kisspeptin 1* and its receptor, which was previously known as *GPR54*). Finally, candidate genes include the GnRH gene itself (*GnRH1*) and its receptor (*GnRHR*).

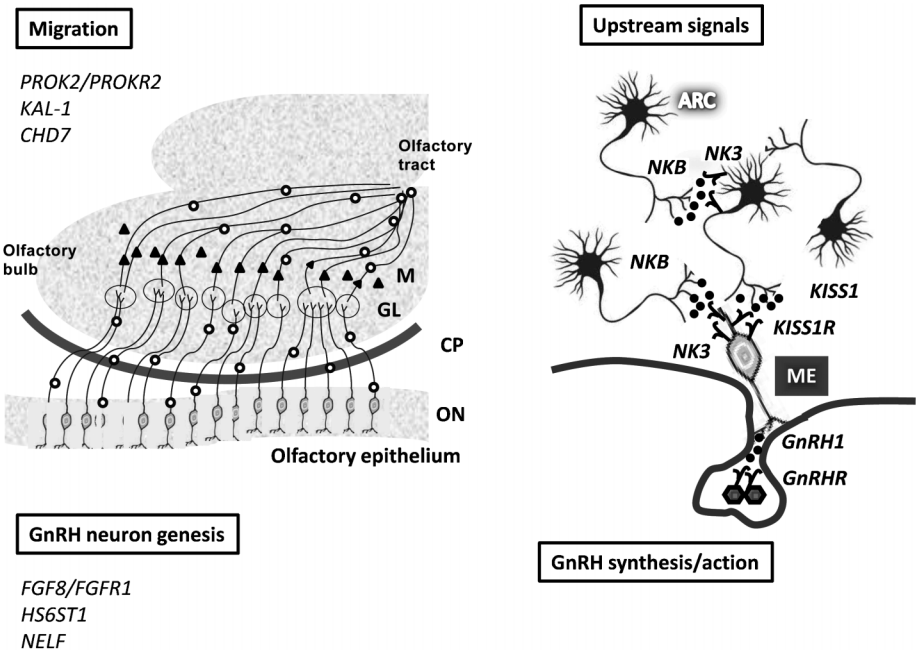

Figure 1 Schematic representation of the genes implicated in HPG activation. In each step, the different genes whose genetic variants disrupt the HPG axis activity are indicated. ARC, arcuate nucleus; CP, cribriform plate; GL, glomerulus; HPG, hypothalamic–pituitary–gonadal; M, mitral cell; ME, median eminence; ON, olfactory neurons.

KAL1

The *KAL1* gene (OMIM number: 308700), which is located on chromosome Xp22.3, consists of 14 exons that encode an extracellular matrix glycoprotein of approximately 100 kDa and is termed anosmin-1. It consists of a cysteine-rich region, a whey acidic protein domain and four fibronectin type III domains.[6,7] The fibronectin type III domains appear to create a flat surface with a strong basic charge, which enables interactions to occur between anosmin-1 and other components of the extracellular matrix.[10,11] Anosmin-1 is expressed in the human forebrain at 5 weeks of gestation at the area of contact with the olfactory fibres and appears to stimulate afferent projections to the olfactory bulb.[12] A post-mortem study of a 19-week-old male foetus with a deletion that included *KAL1* provided important insights into the pathology of X-linked KS; this study revealed both an absence of olfactory bulbs and the abnormal migration of GnRH neurons that had differentiated from the olfactory placode and had begun to migrate before they were arrested in a tangle of olfactory neurons at the point of entry into the central nervous system.[13] Various types of *KAL1* gene abnormalities have been reported in patients with KS. These abnormalities include missense and nonsense mutations, splice site mutations, intragenic deletions and submicroscopic chromosomal deletions involving the entire *KAL1* gene.[14] Clinical features in mutation-positive males include ICH and anosmia/hyposmia. In addition, several non-reproductive and non-olfactory disorders are largely attributed to *KAL1* defects, including midline facial defects, such as cleft lip and/or cleft palate, short metacarpals, renal agenesis, sensorineural hearing loss, bimanual synkinesia, oculomotor abnormalities and cerebellar ataxia.[15] Of these defects, renal agenesis and bimanual synkinesis show the highest incidences; they occur in approximately 30%–40% and 75% of KS cases, respectively.[16] *KAL1* mutations have been detected in approximately 60% of patients with familial KS, which suggests an X-linked mode of inheritance; they have been detected in only 10%–15% of male patients with sporadic KS.[14] Furthermore, nearly all mutations have been identified in patients with both ICH and olfactory dysfunction of variable extents. To date, only one case with nICH and a *KAL1* mutation has been reported in the literature.[17]

FGFR1 and *FGF8*

The *FGFR1* gene (OMIM number: 136 350), which is also called KAL2, is located on chromosome 8p12 and comprises 18 exons. The mature *FGFR1* protein belongs to the receptor tyrosine kinase superfamily, and it consists of four subtypes (*FGFR1–4*) and seven FGFR isoforms, which specifically bind to particular members of the 22 fibroblast growth factor (FGF) ligands. *FGFR1* consists of three extracellular immunoglobulin-like loops (D1, D2 and D3), an acid box that is located between the first two immunoglobulin loops (the D1–D2 linker region that contains a stretch of negatively charged amino acids and a heparan sulphate-binding site within the first half of D2), a transmembrane domain and an intracellular split tyrosine kinase domain.[18] Alternative splicing within the second half of the juxtamembrane D3 domain generates two transmembrane isoforms, IIIb and IIIc.[19] The cognate FGF ligand binding is formed by the proximal membrane D2 and D3 domains. The D3 isoform determines ligand-binding specificity while the D1 domain can fold back to interact with D2 in the FGF and heparan sulphate (HS) contact sites; therefore, it functions as a potential competitive auto-inhibitor of the interaction between FGFR and FGF and HS. *FGFR1* signalling is achieved by the induction of conformational changes in the receptor upon ligand binding, which leads to dimerisation and the subsequent activation by autophosphorylation of its intracellular tyrosine kinase domains. In addition to requiring two FGF ligands, the binding of heparin or HS proteoglycan is also essential for FGF receptor dimerisation and the activation of the FGF–FGFR complex.[20] *FGFR1* signalling has been shown to play a broad role during embryogenesis, homeostasis and wound healing. Several studies on the expression patterns of FGF ligands and receptors during central nervous system development have indicated the critical role of FGF in the initial generation of neural tissue.[21] This activity is also present in the rostral forebrain; it directly affects the olfactory bulb development, which may influence the GnRH neuronal migratory activity.[22] *Fgfr1*$^{-/-}$ knockout (KO) mice show persistent cell proliferation within the anterior end of the telencephalon, and this leads aplasia in the olfactory bulbs.[22] Furthermore, a 30% decrease in the number of hypothalamic GnRH neurons was observed in mice with dominant negative *Fgfr1*$^{-/-}$ mutations that were targeted to GnRH neurons,[23] and the early emergence of GnRH neurons appeared to be disrupted in *Fgfr1*$^{-/-}$ hypomorphic mice.[24] Therefore, loss-of-function mutations in *FGFR1* result in a defect in GnRH neuron migration *via* the abnormal morphogenesis of the olfactory bulb, while specific gain-of-function mutations in *FGFR1* cause craniosynostosis.[25] Dode and Hardelin[16] first reported the association between *FGFR1* loss-of-function mutations and the dominant form of KS. Since that observation, several *FGFR1* mutations, all of which occurred in the *FGFR1IIIc* isoform and spanned all of the functional domains of the receptor, have been identified in KS-affected individuals and in patients with nICH.[26,27] Similar to KAL1 defects, other non-reproductive and non-olfactory disorders, such as cleft palate or lip, dental agenesis and bimanual synkinesis, are associated with KS due to *FGFR1* defects.[15] An important phenotypic variability has been reported in mutated *FGFR1* in unrelated probands and mixed pedigrees. The coexistence of KS, isolated hyposmia without hypogonadism, and nICH was observed even in patients from the same family that shared an identical mutation.[28] The presence of modifier genes, the involvement of multiple genes or an epigenetic mechanism may explain this different phenotypic expression of the same genetic defect.[29] It is therefore evident that the precise quantitative regulation of *FGFR1* signalling is essential for normal development, and both attenuated and increased signalling cause disparate phenotypes.

FGF family ligands consist of 22 structurally related proteins with primary sequence differences that determine the FGF–FGFR-specific interaction and thus regulate the diverse function of the FGF pathway. At least 11 different FGFs can activate *FGFR1*, but *FGF8* has been identified as a key ligand for *FGFR1IIIc* in KS and nICH pathogenesis.[30] Thus, the *FGF8* gene (OMIM number: 600483), which is located on chromosome 10q24, is also called KAL6. This role has been confirmed in mice that are homozygous for an *Fgf8* hypomorphic allele; these mice had small telencephalons with no olfactory bulbs in addition to other cardiac, craniofacial, forebrain, midbrain and cerebellar developmental abnormalities, which included the absence of GnRH neurons.[24,30] Overall, these data demonstrate the crucial role of *FGF8*-signalling activation through the assembly of the FGF8–FGFR1–HS complex in the GnRH neuronal and olfactory system development. Nevertheless, other FGF mutations can impair the FGF–FGFR1 pathway and lead to ICH.

HS6ST1

During the preparation of this manuscript, *HS6ST1*, which is (OMIM number: 604846) located on chromosome 2q21 and involved in extracellular sugar modification, was indeed found to be mutated in patients with ICH.[31] HS polysaccharides are extracellular matrix

components that modulate the cell–cell communications that are important for neural development.[32] This post-translational modification is important for the interaction and activation of the FGFR–FGF complex[20] and for the interaction of anosmin1 with the cell membrane.[10,11,33] The importance of these molecular interactions has been confirmed in a model of transgenic nematodes that mis-/overexpress the *KAL1* orthologue in certain neurons; the axon-branching phenotype was reversed in mutant worms that lacked either *HS6ST1* or C5-epimerase, which is another enzyme that is involved in the biosynthesis of HSPS chains.[34,35] *HS6ST1* appears to be mutated in KS or nICH patients with a wide spectrum of severity and timing of the onset of GnRH deficiency.[31] Clinical variability is also evident both within and across families that carry the same genetic alteration.[31] All of the identified *HS6ST1* variants were also tested *in vivo* and *in vitro*, and they displayed impaired activities in both assays.[31]

PROKR2 and *PROK2*

Another pair of receptor–ligand genes, *PROKR2* (OMIM number: 607123), which is located on chromosome 20p13 and also called KAL3, and *PROK2* (OMIM number: 607002), which is located on chromosome 3p21.1 and also called KAL4, were considered to be potential causative genes of KS. The mutant mice that lacked *PROKR2*, which is a transmembrane receptor interacting with the heterotrimeric guanine nucleotide-binding proteins (G protein-coupled receptor (GPCR)), presented abnormal olfactory bulb development that was combined with severe atrophy of the reproductive system.[36] The prokineticin system is composed of two nearly identical (approximately 85% gene homology) receptors (*PROKR1* and *PROKR2*) and their two polypeptide ligands, *PROK1* and *PROK2*, which show considerably less homology and exhibit quite different anatomic distributions. Although *PROK1* and its receptor, *PROKR1*, are primarily restricted to the gastrointestinal system where they are responsible for motility, *PROK2* and *PROKR2* have a more specific neuroendocrine profile; they are located in the arcuate nucleus, olfactory track and suprachiasmatic nucleus.[37,38] In 2006, Dodé *et al.*[39] demonstrated that mutations in these pairs of ligand–receptor genes cause human GnRH deficiency in patients with nICH or KS. Subsequently, many mutations were described in the literature; most of these mutations are missense mutations, and many are also present in apparently unaffected individuals, which raises questions regarding their pathogenic role in the disease. However, deleterious effects on prokineticin signalling have subsequently been shown *in vitro* for several variations.[40,41] The finding of both heterozygous and homozygous (or compound heterozygous) unrelated patients with *PROKR2* and *PROK2* mutations[39,42] is in favour of a possible oligogenic mode of inheritance in heterozygous patients, which has been demonstrated in some cases.[29,42] A variety of accessory features, including fibrous dysplasia, sleep disorder, severe obesity, synkinesia and epilepsy, have been noted in patients with *PROK2* or *PROKR2* mutations.[40]

KISS1R and *KISS1*

The human *GPR54* gene (OMIM number: 604161) is located on chromosome 19p13.3 and encodes a 398-amino acid GPCR whose ligand binding-induced activation determines, *via* a Gq pathway, the intracellular accumulation of IP3/calcium.[43] *GPR54* has been shown to be the receptor for small peptides that are derived from the *KISS1* gene,[44] which caused its recent redesignation as *KISS1R*. The *KISS1* gene (OMIM number: 603286), which is located on chromosome 1q32, encodes a 145-amino acid precursor peptide, kisspeptin 1, which produces four cleavage products: the longest 54-amino acid peptide, which is known as kisspeptin 54 or metastatin (composed of amino acids 68–121) and three smaller products of 14, 13 and 10 residues (kisspeptin 14, kisspeptin 13 and kisspeptin 10). All four peptides exhibit the same affinity and efficacy towards *KISS1R*, indicating that the C-terminal end of the peptide is responsible for the binding and activation of the receptor.[44,45] Although all four kisspeptin products are biologically active,[46] the *in vivo* relevance of the shorter peptides is still unknown. *KISS1R* is expressed in both the hypothalamus and pituitary, while *KISS1* is expressed only in the hypothalamus.[44] Physiological and pharmacological studies have demonstrated the implication of the *KISS1–KISS1R* complex in the HPG axis. *Kiss1r*$^{-/-}$ KO mice show an ICH phenotype with nearly absent sexual maturation even in the presence of eutopic GnRH-secreting neurons that have a normal GnRH content, which demonstrates that *KISS1R* affects either the processing or release of this hormone but not the migration of GnRH neurons.[47,48] Several studies have demonstrated the ability of kisspeptin to elicit the release of LH and FSH in different animal species[48–54] and in humans.[55] This effect is mediated by the *KISS1–KISS1R* interaction because it is not present in the *Kiss1r* KO mice and is GnRH release-dependent; it is not directly due to kisspeptin action on the pituitary because it is inhibited by the co-administration of GnRH antagonist.[49,50,56] Furthermore, multiple independent mouse knockouts of *Kiss1r* and *Kiss1* have been described, and all of them recapitulate the human nICH phenotype, which confirms that *KISS1/KISS1R* are robust proximal regulators of GnRH release.[57] *KISS1*-secreting neurons are also highly responsive to oestrogen, and they have been implicated in both the negative and positive central feedback of sex steroids to GnRH production.[58–60] In 2003, using linkage mapping in familial nICH, two different groups simultaneously discovered the presence of a loss-of-function mutation in *KISS1R* as genetic cause of nICH.[47,61] Further reports of human loss-of-function mutations in *KISS1R* that were associated with autosomal recessive nICH have been published in recent years, although this genetic cause of nICH appears to be extremely rare.[62] Considering the demonstrated importance of the *KISS1–KISS1R* complex in the control of pubertal onset, the *KISS1* gene is another obvious candidate for genetic screening in cases of ICH. Nevertheless, no pathogenic *KISS1* mutations in association with ICH have been reported to date.

TACR3 and *TAC3*

The application of the strategy of studying nICH in rare consanguineous families with multiple affected members and no mutations in known ICH-related genes led to the identification of three different loss-of-function mutations in *TACR3* (OMIM number: 152332), which is the gene located on chromosome 4q25 that encodes NK3R, and one mutation in *TAC3* (OMIM number: 162330), which is the gene that is located on chromosome 12q13–q21 and encodes NKB, which is the endogenous ligand of NK3R.[63,64] Following these initial studies, defects in either *TACR3* or *TAC3* were found in several patients; this demonstrates that intact function of the *NKB/NK3R* system is required for normal HPG activation at puberty. Moreover, the association of the *TACR3* mutation with micropenis and cryptorchidism in male patients indicates that an intact *NKB/NK3R* function is required for normal foetal gonadotrophin secretion. *NKB* is a member of the tachykinin superfamily of neuropeptides that also includes substance P and neurokinin A.[65] *NK3R*, which is a GPCR, is primarily expressed in the central

nervous system and is the most selective one of the tachykinin receptors; it preferentially binds and is activated by *NKB*.[66,67] The mechanism(s) by which mutations in the NKB pathway exert their effects on central neuroendocrine control of puberty and reproduction in humans is unclear.[62] *NKB* is expressed in the arcuate nucleus of hypothalamus neurons that project to GnRH secreting; here, kisspeptin is also expressed, and both peptides in the arcuate are downregulated by oestrogen. On the basis of these data, it is tempting to consider that kisspeptin and *NKB* play similar roles in relaying feedback from sex steroids to GnRH production. However, other observations seem to indicate important differences in their actions, such as the different ability to stimulate GnRH release in different species (kisspeptin can stimulate this release in almost all species, but *NKB* cannot), or the different phenotypes that have been observed in KO mice models of kisspeptin or *NKB* resistance, which are associated with a classic ICH phenotype[67] or grossly normal fertility,[68] respectively. *NKB* has a wider pattern of central nervous system expression than *KISS1*,[69] which suggests that the arcuate may not be the sole, or even the most important, site at which *NKB* exerts its influence on reproductive function. Moreover, the investigations in mutation-carrying patients at several developmental windows demonstrated that variants in the NKB pathway can profoundly impact the function of the HPG axis in late gestation; however, the effect of these same mutations appears to become attenuated over time with the partial or complete reversal of hypogonadotropism in adult life.

GnRHR and *GnRH*

While the most obvious autosomal candidate gene for GnRH deficiency is the *GnRH* gene itself (OMIM number: 152760), which is located on 8p21–8p11.2, it was only in 2009 that the first genetic defects in the *GNRH1* gene were reported.[70,71] GnRH, which acts *via* the GnRH receptor, is one of the most important factors within the HPG axis, and it has long been the most obvious candidate gene to produce a purely functional defect in its activity. This is in accordance with the phenotype that is apparent in the hypogonadal (*hpg*) mouse experimental model, in which CH is linked to an autosomal recessive mutation in the *GnRH* gene and results in the complete absence of GnRH synthesis.[72,73] *Hpg* mice are sexually infantile, infertile and present a hormone profile that is characterized by low sex steroid and gonadotrophin levels.[73] In addition to their reproductive deficits, these mice present a phenotype that is grossly normal with the exception of abnormal tooth maturation and biomineralisation.[74] Moreover, the reproductive deficits of the *hpg* mice were completely reversed and the GnRH expression was restored by gene therapy.[72] The second factor in this receptor–ligand pair is *GnRHR*, in which genetic defects began to be described 15 years ago in ICH patients.[75] The *GnRHR* gene (OMIM number: 138850), which is located on chromosome 4q21.2, encodes a seven transmembrane-spanning GPCR. The activation of *GnRHR* results in the increased activity of phospholipase C and the mobilisation of intracellular calcium by means of the Gq/G11 group of G proteins. Null *Gnrhr* mice models display a similar phenotype to clinical ICH syndrome. A more severe phenotype was observed in the gene trap *Gnrhr*-null mouse model[76] compared with the *N*-ethyl-*N*-nitrosourea-induced model,[77] and they display small sexual organs with low LH/FSH and sex steroid levels, the failure of sexual maturation, infertility and the inability to respond to exogenous GnRH. Following the initial report in humans, several additional *GnRHR* mutations have been described, including mutations in the transmembrane domains, which significantly impair GnRH binding and/or signalling.[15] These variable genotypes result in a wide phenotypic spectrum that ranges from fertile eunuch syndrome and partial idiopathic hypogonadotropic hypogonadism to the most complete form of GnRH resistance that is characterized by cryptorchidism, micropenis, undetectable gonadotrophins and the absence of pubertal development.[78] Nevertheless, both *GnRH1* and *GnRHR* defects produce classic isolated nICH without any associated developmental defects.[62]

CHD7

CHARGE syndrome (colobomata, heart anomalies, choanal atresia, retardation, genital and ear anomalies) is a multisystem autosomal dominant syndrome that shares overlapping features of ICH and hyposmia with KS. CHARGE syndrome is caused by a mutation in the *CHD7* gene (*chromodomain helicase DNA-binding protein 7*; OMIM number: 608892), which is located on chromosome 8q12.1. A *CHD7* genetic screen in a series of ICH/KS patients demonstrated a mutational prevalence of 6%, which therefore suggested that KS/ICH were mild allelic variants of CHARGE syndrome and might be caused by *CHD7* gene mutations;[79] *CHD7* is also known as KAL5. Further studies have demonstrated that *CHD7* mutations may be present in KS patients who had additional features of the CHARGE syndrome phenotype.[80] Thus, *CHD7* genetic screening may be considered for KS/ICH patients who present special features, such as deafness, dysmorphic ears and/or hypoplasia or aplasia of the semicircular canals.

NELF

The *Nelf* (*nasal embryonic LH-releasing hormone factor*) protein was first isolated in mouse, and the expression patterns of the *Nelf* gene in the olfactory axons and GnRH-secreting neurons during development are consistent with its function as a migratory factor for GnRH neurons.[81,82] However, although rare sequence variants have been reported in KS patients,[29,83–85] no functional studies have been reported thus far.

EBF2

The *Ebf2* gene plays a key role in the neuroendocrine axis as proposed by Corradi *et al.*[86] In *Ebf2*-null mutants, because of the defective migration of gonadotrophin-releasing, hormone-synthesising neurons, the formation of the neuroendocrine axis is impaired; this leads to secondary hypogonadism. To date, no mutations have been reported in the human series of KS/nICH.[87]

CRITICAL ASPECTS OF ICH PHENOMICS AND GENOMICS

ICH has been classically classified into two distinct clinical entities, KS and nICH. However, such separate classification has been questioned in recent years, because these two entities may exist in different relatives within unique familial settings, which therefore supports the idea that they may constitute different phenotypic manifestations of the same genetic defect.[26,28,75,88–90] On the basis of this possibility, a novel idea is that ICH may be considered to be a complex genetic disease that is characterized by variable expressivity, penetrance and modes of inheritance. As in multifactorial complex diseases, the pathogenesis of ICH may include the influence of environmental factors, which may exert epigenetic effects on gene expression, and the concurrent involvement of single nucleotide polymorfisms or other genetic defects in two or multiple interacting genes. Indeed, recent reports of patients who harbour pathogenic rare variants in more than one gene have challenged the long-held view of a strictly monogenic

Table 1 Primary clinical features of the Italian ICH series

	KS	*nICH*	*Total*
Groups (*n* and sex distribution %)	97 (43%) (1 male; 6 females)	130 (57%) (1 male; 4 females)	227 (male: 83%; female: 17%)
Age at diagnosis (years, range)	2–45	13–61	—
Familial cases	20/97 (20.0%)	17/130 (13.0%)	37/227 (16.2%)
Midline defects	12/97 (12.3%)	3/130 (2.3%)	15/227 (6.6%)
Bimanual synkinesis	4/97 (4.1%)	0/130	4/227 (1.7%)
Renal agenesia/aplasia	3/97 (3.1%)	1/130 (0.7%)	4/227 (1.7%)
Hearing defects	2/97 (2.1%)	0/130	2/227 (0.8%)
Cryptorchidism	29/83 (35.0%) (monolateral: 14; bilateral: 15)	11/105 (10.0%) (monolateral: 3; bilateral: 9)	40/188 (21.2%) (monolateral: 17; bilateral: 24)

Abbreviations: ICH, idiopathic central hypogonadism; KS, Kallmann syndrome; nICH, normo-osmic ICH.

disorder. Oligogenicity, which is as frequent as the biallelic defects in a single gene, may partially account for the phenotypic variability of isolated GnRH deficiency.[29] Furthermore, the gene–environment interaction is supported by: (i) the presence of rare variants in genes for isolated GnRH deficiency in unaffected patients, which suggests that such variants may also underlie milder forms of GnRH deficiency when combined with environmental factors, which was recently described in a series of patients with acquired hypothalamic amenorrhea and heterozygous rare variants in *FGFR1*, *KAL1*, *PROKR2* and *GnRHR*;[91] (ii) the occasional adult onset of ICH following normal puberty and reproductive function;[92,93] and (iii) the reversal of ICH in a subset of patients who experience an extremely delayed onset of a spontaneous GnRH function during the withdrawal of steroid administration, which can even occur beyond 25 years of age.[94]

THE ITALIAN NETWORK FOR ICH

Because of the involvement of numerous clinicians from several Italian regions (see the list at the note of first page of the manuscript) and thanks to the support of the Italian Societies of Endocrinology and Paediatric Endocrinology and Diabetes, we were able to collect a large series of patients who are affected with ICH. To date, we have collected a total number of 227 ICH patients, which are 83% males and 17% females and have either KS (43%; 1 female:6 males) or nICH (57%; 1 female:4 males). The most important clinical data are summarized in **Table 1**.

Because genetic investigations that are aimed to reveal the possible genetic cause of ICH in a single case are extremely expensive and tedious and cannot be sustained by a single centre, we decided to coordinate the efforts of several molecular biology laboratories in Italy to set up an extensive service of genetic analyses that are aimed to support counselling of families at risk. Therefore, because of the contribution of the collaborating Italian centres, we began to screen the entire coding regions of the most relevant candidate genes for ICH pathogenesis. The current results are summarized in **Table 2**, and they show the identification of a contributing genetic defect in approximately 35% of the patients. Genetic data from Italian patients show a major involvement of *KAL1*, *FGFR1* and *PROKR2*, and the rare involvement of variants in the other candidate genes. Compared with the data that have been reported in the literature, our series seem to show a higher percentage of genetic alterations in the *FGFR1* and *PROKR2* genes and a lower percentage in the *GnRHR* gene in the nICH patients (see data in **Table 2** and in Ref. 62). Of note, we have not yet identified any alterations in the coding regions of *GNRH1*, *KISS1R*, *TAC3* and *TACR3*, although the screening of these genes was so far accomplished in only a part of the entire cohort. Interestingly, in our series, a digenic involvement was detected in three cases. Overall, these data indicate that we can support genetic counselling for approximately one-third of the affected families. Such an outcome may be considered to be valuable because it can lead to the early recognition and prepubertal treatment of several ICH cases. Nevertheless, the genetic studies were negative in approximately 65% of the patients, which is similar to the results that have been reported in the literature.

FUTURE PERSPECTIVES

Because two-thirds of ICH patients lack a clear molecular basis, new strategic and methodological advances are needed to cover this gap. The likely involvement of still-unknown candidate genes may be identified by the use of exome sequencing or genome-wide investigation techniques. Moreover, the contributions of cryptic genetic defects that are unresolved by direct sequencing or those of alterations in non-genetic mechanisms that regulate gene expression (i.e., abnormal states of gene methylation or micro-RNA defects) still remain to be elucidated. Nevertheless, the accumulation of large consortia is required to cover the elevated costs and collect sufficiently numerous cohorts to increase the success rate of these experiments that investigate the whole genome or the regulatory mechanisms without any prior selection.

COMPETING FINANCIAL INTERESTS

The authors declare no conflict of interest related to this work.

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Table 2 Results of the analyses of the principal candidate genes in the Italian ICH series

	Fgfr1	*Fgf8*	*Prok2*	*ProkR2*	*Kal1*	*GnRHR*	*GnRH1*	*KissR1*	*Tac3*	*TacR3*	*Ebf2*
KS	8/77	1/23	2/95	10/95	6/64	0/43	0/37	0/40	0/5	0/5	0/21
nICH	9/110	2/57	2/129	7/129	—	4/58	0/56	0/53	0/13	0/13	—
Total	17/187 (9%)	3/80 (3.7%)	4/224 (1.7%)	17/224 (7.5%)	6/64 (9.3%)	4/101 (3.9%)	0/93	0/93	0/18	0/18	0/21

Abbreviations: ICH, idiopathic central hypogonadism; KS, Kallmann syndrome; nICH, normo-osmic ICH.

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