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Review

GnRH receptor mutations in isolated gonadotropic deficiency

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ABSTRACT

GnRH and its receptor GnRHR are key regulators of the hypothalamo–pituitary axis. They modulate the secretion of LH and FSH gonadotropins and therefore, the development and maturation of gonads in fetal life as well as after birth. Congenital functional defect of this axis results in isolated hypogonadotropic hypogonadism (IHH). Several natural mutations causing IHH without anosmia have now been identified in GnRHR or GnRH genes. These mutations inactivate GnRHR or its ligand function and cause highly variable phenotypes, ranging from partial to complete gonadotropic deficiencies. The present review describes the published natural GnRHR mutations and tries to correlate them with the corresponding phenotypes according to the different steps of the GnRH system development.

Contents

1. The GnRH system: an early functional hypothalamo–pituitary system during development

The GnRH system is composed of a ligand–receptor pair which regulates the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by anterior pituitary cells (Plant, 2008). LH and FSH act on gonads to regulate estrogen and testosterone synthesis as well as gametogenesis. GnRH is a highly conserved decapeptide encoded by the GnRHI gene located on chromosome 8p11.2-p21. This peptide is produced by a proconvertase cleavage of the preprohormone into the mature decapeptide and the GnRH associated peptide and by an amidation of the carboxyl end. GnRH is secreted by specific hypothalamic neurons projecting axonal extremities toward the median eminence, where GnRH is released into the hypothalamo–pituitary portal system. GnRH neurons ontogenesis is complex and specific. Indeed, GnRH neurons emerge from the olfactory placode around the 5th week of gestation and begin to migrate the next week along the nervus terminalis fibers and the olfactory tract to reach the hypothalamus (Schwanzel-Fukuda et al., 1996; Wray, 2010) (Fig. 1). Their development is therefore highly associated with the normal development of olfactory bulbs (Cariboni and Maggi, 2006). In the meantime occurs the development of gonadotropes, which are pituitary cells secreting LH and FSH (Winter et al., 1977). The hypothalamo–pituitary unit becomes functional around the 15th week of gestation in Human. It is absolutely required in males for the normal growth of primary sexual characteristics during the second

Abbreviations: GnRH, gonadotropin-releasing hormone; GnRHR, gonadotropin-releasing hormone receptor; LH, luteinizing hormone; FSH, follicle-stimulating hormone; IHH, idiopathic hypogonadotropic hypogonadism; nIHH, normosmic idiopathic hypogonadotropic hypogonadism; GPCR, G protein-coupled receptor; PLC, phospholipase C; IP3, inositol-(1,4,5)-triphosphate; ER, endoplasmic reticulum.

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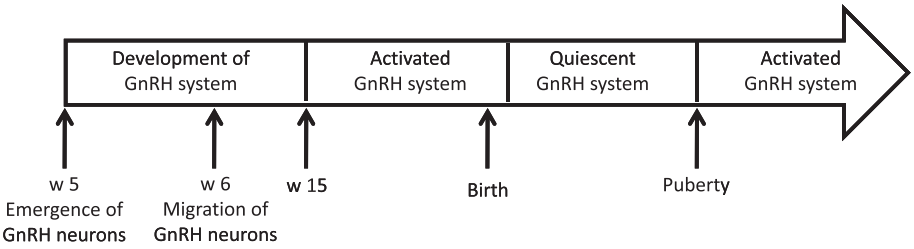

Fig. 1. Development and activation of the GnRH system. GnRH neurons emerged from the olfactory placode around the 5th week of gestation and migrate from the nasal epithelium toward the hypothalamus. GnRH system was first activated around the 15th week, became quiescent just after the birth and was then active at the onset of puberty. w: week of gestation.

and the third trimesters of pregnancy. It also participates in the normal development of gametes in both males and females (Winter et al., 1977). The GnRH bioactivity is therefore crucial for normal fetal development of gonads and sexual organs.

After birth, the GnRH system is temporally activated by unknown mechanisms and short as well as long term consequences of this activation remain unclear. Sexual hormones and gonadotropins plasmatic levels observed during this post-natal activation are almost equivalent to those observed in adults (Waldhauser et al., 1981). During childhood, the gonadotropic axis becomes quiescent, with low levels of plasmatic LH and FSH due to inhibitory inputs on hypothalamic neurons (Forest et al., 1976). The responsiveness of gonadotropes to GnRH during this period is very low and partly reflects a reduced expression of the GnRH receptor (GnRHR) at the cell surface.

Puberty marks the waking up of the gonadotropic axis. LH and FSH synthesis and secretion concomitantly increase with the GnRH release by the hypothalamus (Grumbach, 2002). At the end of puberty, the GnRH system is completely mature in both males and females to ensure a normal reproductive function.

The neuroendocrine regulation of the GnRH hypothalamic release is quite complex and results from an interplay of activating and inhibitory inputs. A major advance in the understanding of these mechanisms was recently made when the fundamental role of hypothalamic kisspeptins and their receptor KISS1R (previously named GPR54) on the GnRH secretion was described (for an extensive review, see d'Anglemont de Tassigny and Colledge, 2010). The regulation of GnRH release by kisspeptins is crucial for the gonadotropic axis functional integrity during fetal development and pubertal onset and to maintain fertility in adults. Kisspeptins are the hypothalamic mediators of both negative and positive feedbacks of estrogens (E2) on the gonadotropic axis. Moreover, kisspeptin secreting neurons are actually considered as the master neuroendocrine center of the gonadotropic axis, through which converge the majority of negative and positive regulators of the gonadotropic axis. The integrity of the kisspeptin/KISS1R system is therefore fundamental to ensure a normal reproductive function.

The fetal development of the hypothalamo–pituitary axis and GnRH neurons migration is similar in both males and females. However, in adult rodents, GnRH neurons are sexually dimorphic (Ciofi, 2000). In males, the period and the amplitude of the GnRH secretion pulsatile peak are relatively constant throughout the adult life, the latter being directly related to testosterone plasmatic levels. In females, the situation is more complex because of variations in the GnRH pulsatile release throughout the ovarian cycle. Indeed, at the end of the follicular phase a large GnRH secretory peak leads to a massive release of LH. The increase of GnRH is induced by a positive feedback of E2 on kisspeptin neurons. The regulation of GnRH release is therefore cyclic in females while it is tonic in males. The molecular mechanisms underlying the sexual dimorphism of the neuroendocrine regulation of the gonadotropic axis and the exact timing of its emergence remain unknown.

2. The GnRH system: a natural candidate system to explain isolated hypogonadotropic hypogonadism

This brief description of the GnRH system dynamic regulation highlights the fact that the integrity of the modulation of the GnRH bioactivity is crucial during pre-natal development, birth, puberty and adulthood. The GnRH system has rapidly emerged as the best candidate to explain congenital isolated hypogonadotropic hypogonadism (IHH). This pathological condition is defined by low sexual hormones plasmatic concentrations associated with low or normal concentrations of LH and FSH plasmatic levels. The hormonal deficit is isolated and not syndromic because all other hormonal axis are normal, and no clinical symptom except those related to hypogonadism are observed. For instance, there is no anosmia, no neurological phenotype and no abnormal facial development like cleft palate, which is frequently found in Kallmann syndrome. Normosmic IHH (nIHH) therefore is an endocrine congenital disease due to a functional defect of the hypothalamo–pituitary unit, which may be related to abnormal synthesis, secretion or altered capacity of GnRH to induce gonadotropins synthesis and release.

Segregation analysis in informative families initially identified congenital IHH as a monogenic Mendelian disease (for review, see Seminara et al., 1998). Since the description of GnRH gene deletion in HPG mice in the early 90s (Mason et al., 1986), GnRH has rapidly emerged as a natural nIHH candidate gene. At that time, the rudimentary molecular techniques available to verify this hypothesis were very time-consuming. Few abstracts even reported negative results, suggesting a very low frequency of GnRH mutation in nIHH (Layman, 1999; Weiss et al., 1991). Almost 20 years were in fact necessary before two groups finally reported that GnRH inactivating mutations caused nIHH in humans (Bouligand et al., 2009; Chan et al., 2009). The observed phenotype in these cases was an isolated gonadotropic deficiency without anosmia. In the first family, the genetic defect was a homozygous insertion of an adenine at nucleotide 18 (c.18-19InsA), leading to a coding sequence frameshift and therefore an absence of GnRH synthesis (Bouligand et al., 2009). As expected for such total invalidation of GnRH synthesis, the two siblings had a complete gonadotropic deficiency with a microphallus, testicular cryptorchidism, complete impuberism and very low gonadotropins and steroid hormone levels. The second case was a prepubertal boy with a severe congenital nIHH caused by a frameshift deletion at the nucleotide 87 (c.87delA) leading to a premature stop codon and a truncated GnRH form (Chan et al., 2009). GnRH1 mutations frequency is very low in European (1/150) (Bouligand et al., 2009) as well as in US (1/310) patients (Chan et al., 2009). Four heterozygous missense and nonsense point mutations of GnRH1 were also described in nIHH patients (Chan et al., 2009) but the exact contribution of these variants to the gonadotropic deficiency has not yet been established.

The GnRH "null mutation" phenotype recapitulates the most severe form of gonadotropic deficiency due to GnRHR loss of

function mutations. During the mid-90s, a new concept of "natural mutations of G protein-coupled receptors (GPCR)" leading to endocrine diseases was verified for several receptors. This concept was based on the observation that point mutation of GPCR may induce loss of function as well as constitutive activation of these receptors (Allen et al., 1991; Dryja et al., 1990). Loss of function would therefore result in hormonal deficiency while constitutive mutations would result in an increase of hormonal secretion. Furthermore, it quickly appeared that inactivating mutation of GPCR did not only result in complete hormonal deficiency but also in partial endocrine deficit (de Roux et al., 1996). We applied this concept of GPCR natural mutations to nIHH patients with partial or complete gonadotropic deficiency. This strategy led to the description of the first nIHH case of compound heterozygote GnRHR inactivating mutation in two siblings from unrelated parents (de Roux et al., 1997). This result opened the field of nIHH genetics and was then followed by the discovery of kisspeptins and more recently of neurokinin B as major regulators of the GnRH pulsatile release.

3. Loss of function mutations of the GnRH receptor: what we have learned on the structure–function of the receptor

Describing natural mutations of GPCR family was initially considered as an interesting strategy to point out consensus functional regions important for normal *in vivo* activation. This strategy was therefore considered as an informative way to analyze GPCRs structure–function relationships. The characterization of mutations in constitutively active unliganded GPCR has indeed been very powerful to modelize receptor activation. Therefore, 12 years after the first description of GnRHR mutations, structure–function analysis of GnRHR natural mutations efficiently contributed to the understanding of GnRHR biochemistry. This strategy is complementary to *in vitro* mutagenesis analysis (for an extensive review on this point, see Millar et al., 2004). Natural GnRHR mutants also led to a better understanding of the endoplasmic reticulum (ER) quality control mechanism allowing only correctly folded protein to enter the secretory pathway (Brothers et al., 2004). The concept that misfolded GPCR cannot pass the quality control was initially described for the rhodopsin (Kaushal and Khorana, 1994). It was then extended to the vasopressin type 2 receptor (Morello et al., 2001) and to the GnRH receptor (Brothers et al., 2004).

Human GnRHR is encoded by a 3 exons gene located on chromosome 4 (Kaiser et al., 1994). It is a 328 amino acids protein folded in seven transmembrane domains linked by extracellular and intracellular loops. The extracellular domain is a 38 residues sequence comprising one of the two glycosylation sites at residue N18. The other resides in the second extracellular loop at position N102 (Kakar et al., 1992). The glycosylation status of rodent GnRHR seems important for a normal expression (Davidson et al., 1995, 1996) but natural mutation affecting the glycosylation status of the human GnRHR has never been described. The main distinctive feature of the human GnRHR among the GPCR family is the lack of a C-terminal cytoplasmic domain (Kakar et al., 1992), which is normally involved in the intracellular trafficking during the exporting pathway or after receptor activation.

Up to 22 mutations of the human GnRHR have now been described, all distributed along the protein, in the transmembrane domains and extracellular or intracellular loops (see Table 1 and Fig. 2). The Gln106Arg, which is located in the first extracellular loop, is the most frequent mutation (Beranova et al., 2001; Costa et al., 2001; de Roux et al., 1997, 1999; Karges et al., 2003; Kottler et al., 2000; Pitteloud et al., 2001). In all published cases of GnRHR loss of function mutations to date, nIHH is transmitted as a recessive trait. Affected patients are therefore homozygote for one mutation or compound heterozygote for two mutations, each on one allele. In rare sporadic nIHH cases, heterozygous mutations were identified (Sykiotis et al., 2010; Vagenakis et al., 2005 and our experience). However, we never observed any phenotype in heterozygote parents or siblings of nIHH patients bearing homozygous or compound heterozygote inactivating GnRHR mutations. This recessive transmission confirms the genetic model observed in GNRHR knock-out mouse (Wu et al., 2010). A second genetic

Table 1
Location and function of GnRHR mutations identified in IHH patients.

Mutation	Location	Cell surface expression[a]	Binding	PLC pathway	Genes transactivation (LHβ, αLuc, FSHβ, GnRHR)	IN3 rescue	References
Asn10Lys	N-ter	+	↑Kd	↓	nd	Yes	1, 19
Asn10Lys + Gln11Lys	N-ter	+	↓Bmax	+	+	nd	2
Thr32Ile	Nter-TM 1	+	–	↓	↓	Yes	3, 4, 19, 20
Glu90Lys	TM 2	nd	–	–	nd	Yes	5, 6, 19, 20
Thr104Ile	EL 1	nd	nd	nd	nd	nd	7
Gln106Arg	EL 1	nd	↓	↓	nd	Yes	8, 19
Tyr108Cys	EL 1	nd	nd	nd	nd	nd	7
Ala129Asp	TM 3	nd	–	–	nd	No	9, 21
Arg139Cys	DRS motif (TM 3–IL 2)	nd	–	–	nd	Yes	10
Arg139His	DRS motif (TM 3–IL 2)	+	–	–	nd	Yes	1, 19
Pro146Ser	IL 2	nd	nd	nd	nd	nd	11
Ser168Arg	TM 4	+	–	–	nd	No	12
Ala171Thr	TM 4	+	–	–	nd	Yes	13, 21
Cys200Tyr	EL 2	+	–	–	↓	Yes	3, 4, 19, 20
Ser217Arg	TM 5	nd	–	–	nd	No	14
Arg262Gln	IL 3	nd	+	↓	nd	Yes	8, 19
Leu266Arg	EL 3	+	–	–	–	Yes	3, 4, 19, 20
Cys279Tyr	TM 6	+	–	–	–	Yes	3, 4, 19, 20
Tyr284Cys	TM 6	nd	↓Bmax	↓	nd	Yes	15, 16, 19
Leu314X	TM 7	nd	–	–	nd	No	17, 21
Pro320Leu	TM 7	+	–	–	–	nd	2
Exon 2 deletion		nd	nd	nd	nd	nd	18

nd: not defined; +: comparable with WT-GnRHR; –: no measurable effect; ↓: decrease compared to WT-GnRHR; [a]: studied by immunocytochemistry of tagged-GnRHR; TM: transmembrane domain; EL: extracellular loop; IL: intracellular loop.
1: Costa et al. (2001); 2: Meysing et al. (2004); 3: Beranova et al. (2001); 4: Bedecarrats et al. (2003a); 5: Soderlund et al. (2001); 6: Maya-Nunez et al. (2002); 7: Antelli et al. (2006); 8: de Roux et al. (1997); 9: Caron et al. (1999); 10: Topaloglu et al. (2006); 11: Vagenakis et al. (2005); 12: Pralong et al. (1999); 13: Karges et al. (2003); 14: de Roux et al. (1999); 15: Layman et al. (1998); 16: Layman et al. (2001); 17: Kottler et al. (2000); 18: Silveira et al. (2002); 19: Leanos-Miranda et al. (2002); 20: Janovick et al. (2002); 21: Leanos-Miranda et al. (2002).

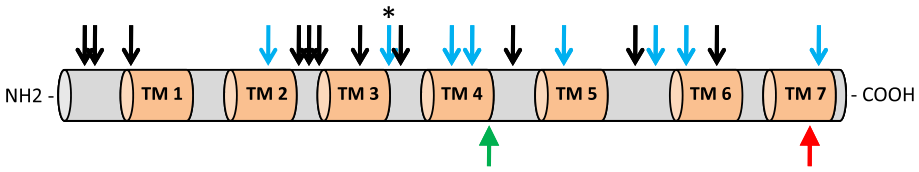

Fig. 2. Distribution of GnRHR mutations along the entire sequence of the receptor. The 22 GnRHR mutations identified to date were all along the receptor. The majority of GnRHR mutations are missense mutations. Black arrow: missense mutation with partial loss of function; blue arrow: missense mutation with total loss of function; gray arrow: mutation in the acceptor splice site of exon 2; red arrow: nonsense mutation; ∗: two different missense mutations identified in the same position.

event in a gene encoding a protein directly involved in the GnRH biochemical pathway or in the development of GnRH neurons is therefore highly probable in patients bearing GnRHR mutation. Indeed, it appears that some IHH could have an oligogenic inheritance associating GnRHR and FGFR1 mutations (Sykiotis et al., 2010). Two GnRHR mutations (Gln106Arg and Arg262Gln) were described in this paper as associated to FGFR1 mutations leading to a digenic transmission of the gonadotropic deficiency. In our experience of 269 patients who have been re-sequenced for GnRHR, we observed 17 patients bearing compound heterozygote or homozygote GnRHR mutations. A unique heterozygote GnRHR Gln106Arg mutation was observed in three patients with a minor allele frequency of 0.5% (Leka and de Roux, unpublished results). This frequency is quite similar to the frequency observed in normal controls (Sykiotis et al., 2010). In these three patients, we did not observe any mutations in KAL1, Prok2, ProkR2 and FGFR1 (Leka and de Roux, unpublished results). A *GnRHR* heterozygote mutation in association with mutations in an unknown gene may therefore contribute to the occurrence of gonadotropic deficiency in few patients as stated by Sykiotis et al. (2010).

Ligand induced signal transduction of GnRHR in pituitary is highly complex. GnRHR is coupled to Gq/11 protein via its third intracellular loop. Stimulation of GnRHR induces phospholipase C (PLC) activation, which hydrolyses phosphatidyl inositol biphosphate (PIP2) into inositol-(1,4,5)-triphosphate (IP3) and diacyl glycerol (DAG). IP3 triggers intracellular Ca^{2+} mobilization and activation of protein kinase C (PKC), leading to MAP kinases phosphorylation such as p38, ERK and MEK. Activated ERK translocates into the nucleus to transactivate gonadotropins genes (Liu et al., 2002; Naor, 2009). After activation, GPCR C-tail is generally phosphorylated by GRK (G protein-coupled receptor kinase), leading to G protein decoupling from the receptor, which is internalized via the recruitment of β-arrestin. However, this desensitization mechanism does not occur after GnRHR activation by agonist due to its lack of a C-tail domain (Pawson et al., 2008). The internalization and recycling of GnRHR is likely to be different from those observed for other GPCR.

All the 22 described natural mutations of GnRHR contribute to a decrease or a loss of receptor function. Among these, only two lead to the formation of a putative truncated receptor: a nonsense mutation at position 314 (Leu314X) in the seventh transmembrane domain (Kottler et al., 2000) and a mutation in the acceptor splice site of exon 2 leading to an intronic retention (Silveira et al., 2002). The others are missense mutations leading to one amino acid substitution. *In vitro* studies indicate that mutations can alter cell surface expression, GnRH binding or GnRH-induced intracellular signalization. Functional analyses have mainly consisted in measuring competitive binding between radioactive and cold ligands on intact cell surface, IP3 intracellular production or cell surface expression of a fluorescent protein fused to the C-terminal end of the receptor or a TAG at the N-terminal end of the receptor. An effect of some GnRHR mutations on GnRH-induced LHβ and FSHβ promoter transactivation has also been reported (Bedecarrats et al., 2003a; Meysing et al., 2004). To date, no *in vitro* studies regarding Tyr104Ile, Tyr108Cys (Antelli et al., 2006), and Pro146Ser (Vagenakis et al., 2005) mutations have ever been performed. The heterozygous Pro146Ser mutation was identified in two IHH sisters. The mother also carried the mutation but had no GnRH deficiency, therefore suggesting a non-contribution of this mutation to the siblings phenotype (Vagenakis et al., 2005).

Functional analysis has led to the classification of natural mutations in two groups: partial and complete loss of function mutations (Fig. 2). The major interest in partial mutations characterization resides in the possibility to correlate defects between GnRH-induced intracellular signaling pathways and gonadotropin release. Seven GnRHR mutations induce a partial loss of function: Asn10Lys (Costa et al., 2001), Asn10Lys + Gln11Lys (Meysing et al., 2004), Thr32Ile (Bedecarrats et al., 2003a; Beranova et al., 2001), Gln106Arg (de Roux et al., 1997), Cys200Tyr (Bedecarrats et al., 2003a; Beranova et al., 2001), Arg262Gln (de Roux et al., 1997), Tyr284Cys (Layman et al., 1998, 2001). Some of these alter GnRH binding at the cell surface (Asn10Lys (Costa et al., 2001), Asn10Lys + Gln11Lys (Meysing et al., 2004), Thr32Ile (Bedecarrats et al., 2003a; Beranova et al., 2001), Gln106Arg (de Roux et al., 1997), Cys200Tyr (Bedecarrats et al., 2003a; Beranova et al., 2001), Tyr284Cys (Layman et al., 1998, 2001)), whether by decreasing GnRHR affinity for the ligand (Asn10Lys (Costa et al., 2001), Gln106Arg (de Roux et al., 1997)) or by decreasing the receptor expression at the cell surface, as shown by the low Bmax suggesting an altered intracellular trafficking (Asn10-Lys + Gln11Lys (Meysing et al., 2004), Tyr284Cys (Layman et al., 1998, 2001)). Cell surface expression of GnRHR mutants was studied by immunocytochemistry of a tagged-receptor, which is not quantitative. Interestingly, *in vitro* studies of the Cys200Tyr mutant showed no detectable binding at the cell surface, but a faint ability to transactivate gonadotropins genes under high concentration of GnRH (Bedecarrats et al., 2003a).

Because Gln106Arg and Arg262Gln are the most common mutations, their effects on GnRHR function were reported by several groups (Beranova et al., 2001; Caron et al., 1999; Costa et al., 2001; de Roux et al., 1997, 1999; Karges et al., 2003; Kottler et al., 2000; Layman et al., 1998, 2001, 2002; Pitteloud et al., 2001). They represent an informative example on the complexity of GnRHR intracellular signaling. The Gln106Arg substitution is in the first extracellular loop while the Arg262Gln substitution is in the third (de Roux et al., 1997). They often were associated with other heterozygous mutations (Beranova et al., 2001; Caron et al., 1999; Costa et al., 2001; de Roux et al., 1997, 1999; Karges et al., 2003; Kottler et al., 2000; Layman et al., 1998, 2001, 2002) but homozygote patients have also been reported (Beranova et al., 2001; Lin et al., 2006; Pitteloud et al., 2001). *In vitro* studies indicated that both GnRHR mutants were present at the plasma membrane and displayed a similar reduction of normal GnRH-induced PLC activation and IP3 production (Bedecarrats et al., 2003b; de Roux et al., 1997). Further analysis revealed that Gln106Arg induced a decrease of ligand binding, whereas Arg262Gln affected transduction signal without loss of ligand binding (Bedecarrats et al., 2003b; de Roux et al., 1997). The latter observation is in agreement with the role of the third intracellular loop in G protein-coupling and signal transduction. These mutants also have

similar effects on FSHβ and LHβ promoters transactivation (Bedecarrats et al., 2003b). However, ERK activation and transactivation of αGSU and GnRHR promoters are higher in cells transiently expressing the Gln106Arg mutant (Bedecarrats et al., 2003b). Two natural GnRHR mutations leading to similar *in vivo* gonadotropic deficiency may thus differently alter the GnRH-induced intracellular pathways.

Characterization of GnRHR complete loss of function mutations brought further insight into the *in vivo* phenotype of GnRHR invalidation. Nine GnRHR mutations appear to completely abolish GnRHR-induced PLC activation. The Glu90Lys mutant loss of function is due to its absence at the plasma membrane (Maya-Nunez et al., 2002; Soderlund et al., 2001). Although present at the cell surface, other mutants are not able to bind the agonist, for example: Ala171Thr in the fourth transmembrane domain (Karges et al., 2003), Leu266Arg in the third extracellular loop, or Cys279Tyr in the sixth transmembrane domain (Bedecarrats et al., 2003a)). Two homozygous mutations leading to a complete gonadotropin inactivation have been identified in two girls: Arg139His and: Arg139Cys (Costa et al., 2001; Topaloglu et al., 2006), and in a boy Ser168Arg (Pralong et al., 1999). This complete GnRHR loss of function leads to a phenotype very similar to what was recently described in GnRHR invalidated mouse (Wu et al., 2010). It has definitively confirmed that GnRHR activation is primordial for penis and testes normal fetal growth and that pituitary is unable to produce LH and FSH in absence of a functional GnRHR.

4. Loss of function mutations of the GnRH receptor: an informative model to study the quality control of GPCR folding

In eukaryotic cells, ER is the first step compartment of the secretory pathway. It ensures the correct synthesis, modification and delivery of proteins to their proper target sites (Buchberger et al., 2010). Proteins adopt their native conformation in the ER. Therefore, it is the first site where misfolded proteins can be recognized by the quality control of cell machinery to prevent their exit from the ER and therefore their entry into the secretory pathway (Kopito and Ron, 2000). Mutation-induced misfolded proteins are recognized and retained in the ER and eventually routed to the proteasome degradation pathway after polyubiquitination (Buchberger et al., 2010). This disease mechanism has now been described for a large amount of cell surface proteins (Dobson, 2001). Natural mutations causing ER retention of misfolded GPCR were initially reported for mutant Rhodopsin and thereafter for several other receptors, including GnRHR (Brothers et al., 2004; Dobson, 2001).

Several natural GnRHR mutants with reduced GnRH binding were in fact poorly expressed at the cell surface (Leanos-Miranda et al., 2002). These mutations induced a misfolding of the protein, leading to its retention in the ER (Brothers et al., 2004). Pretreatment with IN3, a membrane permeant non-peptidic GnRHR antagonist, increased intracellular signaling and cell surface GnRH binding of several GnRHR mutants: Asn10Lys, Thr32Ile, Gln106Arg, Cys200Tyr, Arg262Gln, Tyr284Cys (Janovick et al., 2002; Leanos-Miranda et al., 2002). It also partially restored ligand-induced binding and intracellular signalization for GnRHR mutants previously described as fully inactive: Glu90Lys, Arg139His, Arg139Cys, Leu266Arg, and Cys279Tyr (Janovick et al., 2002; Leanos-Miranda et al., 2002, 2005; Topaloglu et al., 2006). This result indicated that several GnRHR natural mutations caused the receptor to misfold, but without completely abolishing their capacity to activate the PLC pathway. Interestingly, IN3 rescue was not observed for Ser168Arg, Ser217Arg, Leu314X, and Ala129Asp (Leanos-Miranda et al., 2002, 2005), indicating that the molecular mechanisms underlying low GnRHR expression at the cell surface are not uniform. These mutations may also directly affect the function of the receptor. The difference between total loss of function mutant (Ser168Arg, Ser217Arg, Leu314X, and Ala129Asp) with receptors that could be rescued by pharmacochaperones (Glu90Lys, Arg139His, Arg139Cys, Leu266Arg, and Cys279Tyr) was an additional criterion in the classification of natural mutations of the GNRHR. The quality control process of protein folding initially described for mutant GnRHR was suspected to also be involved in the cellular trafficking of the wild type GNRHR. Indeed, the wild type receptor is faintly expressed at the cell surface. In transiently transfected cells, long-term treatment by IN3 induced a 6-fold increase in GnRHR cell surface expression by facilitating its exit from the ER and its transport toward the plasma membrane (Finch et al., 2008, 2010). This indicated that a pool of cytoplasmic functional GNRHR can reach the plasma membrane under the effect of pharmacological chaperones.

5. Loss of function mutations of the GnRH receptor cause highly variable gonadotropic deficiencies

All GnRHR mutants have been identified in IHH patients only, none having ever been described in Kallmann syndrome patients. As mentioned in the beginning of this review, the GnRH system becomes functional around the 15th week of gestation and is necessary for the normal development and growth of gonads and sexual organs. Micropenis and bilateral cryptorchidism are marks of fetal hypogonadism and may thus reveal gonadotropin deficiency in males at birth. The gonadotropic deficiency may then be confirmed by measuring LH, FSH and testosterone plasmatic concentrations. In females, there is no clinical phenotype at birth, but as in males, gonadotropic deficiency may be biologically measured. However, patients bearing GnRHR mutations reported to date have mostly been referred for pubertal delay or infertility.

The phenotype and therefore the gonadotropic deficiency due to GnRHR mutations are highly variable. Most patients are compound heterozygotes (Fig. 3), the phenotype thus results from a combination of two different mutations. In these cases, correlating the genotype with the phenotype is more difficult, as illustrated by the fact that two identical mutations partly inactivating GnRHR may be associated to partial as well as complete gonadotropic deficiencies (Caron et al., 1999; de Roux et al., 1999; Lin et al., 2006). For example, the Gln106Arg mutant leads to a partial nIHH when associated with the Arg262Gln mutant (de Roux et al., 1997), but leads to complete nIHH when associated with the non-functional Leu314X mutant (Kottler et al., 2000). It was surprising that two compound heterozygote mutations, partially altering the function of the receptor for one (Gln106Arg) or completely inactivating the receptor for the other (Leu314X), resulted in a complete gonadotropic deficiency. This result suggested functional interactions between both mutant receptors and was further confirmed by the report of a dominant negative effect of the truncated Leu314X mutant on the Gln106Arg mutant (Leanos-Miranda et al., 2005). This dominant negative effect is not isolated as it was also reported for several others pairs of mutants (Leanos-Miranda et al., 2005). However, these are *in vitro* results and have never been confirmed *in vivo*. It is absolutely unquestionable that the majority of heterozygote patients for one GnRHR inactivating mutation are unaffected. In some patients, modifier genes as well as environmental factors may influence the severity of the gonadotropic deficiency. This hypothesis is actually supported by the high variability of the gonadotropic deficiency between related patients carrying the same mutations (Caron et al., 1999; de Roux et al., 1999; Lin et al., 2006). Delineating how these modifier genes can affect the phenotype remains a challenge for the next future. One may also suspect that functional complementation between binding-deficient and signaling-deficient GnRHR may also occur, as it was

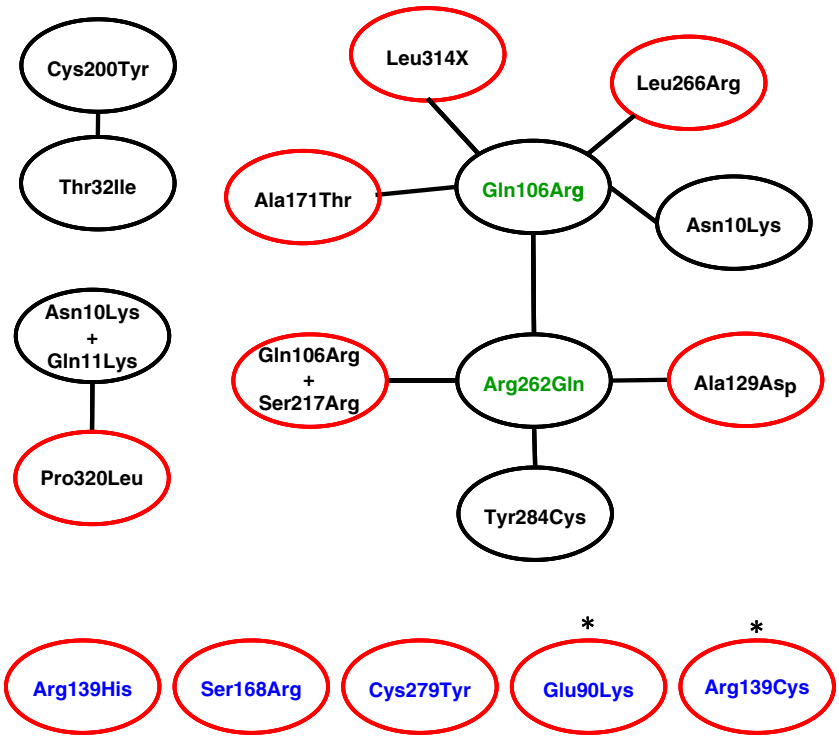

Fig. 3. Association between the different GnRHR mutations identified in IHH patients. Homozygous GnRHR mutations are indicated in blue, mutations identified as homozygous or compound heterozygote are indicated in green and heterozygous mutations are indicated in black. Black circle represents mutation with partial loss of function, red circle represents mutation with total loss of function. Solid line connects compound heterozygous mutations. * represents patients, which have been identified in consanguineous families. Only the mutations for which functional studies have been made are presented in this figure.

recently demonstrated for the LH/CG receptor in transgenic mice (Rivero-Muller et al., 2010).

Nevertheless, the severity of the gonadotropic deficiency is somehow related to the degree of GnRHR loss of function. All homozygous GnRHR mutations inducing a total loss of function are associated with complete IHH (Fig. 3). Two of these five homozygous patients have been identified in a consanguineous family, while no information is known for the three others. According to the very low frequency of these mutations, unknown consanguinity can be suspected. The fertile eunuch syndrome is defined by normal testicular size and some degree of spermatogenesis. This partial gonadotropic deficiency is caused by GnRHR mutations which do not completely abolish the receptor function (Beranova et al., 2001; de Roux et al., 1999; Pitteloud et al., 2001). A reversible phenotype was even reported in a very few adult patients and this phenotype was again caused by partially functional GnRHR mutant (Dewailly et al., 2002; Pitteloud et al., 2001). The screening of GNRHR mutation in a patient suffering from constitutional delay in growth and puberty (CDGP) identified the Arg262Gln mutation in a young male at the age of 16 (Lin et al., 2006). After 6 months of testosterone treatment, puberty spontaneously continued to progress (Lin et al., 2006) and was finally achieved at the age of 18. It must be mentioned that his younger brother had a persistent hypogonadotropic hypogonadism (Lin et al., 2006). A screening in a well defined population of subjects with late puberty did not reveal any GNRHR mutation (Sedlmeyer et al., 2005). These results bring more arguments in favor of distinct molecular mechanisms between nIHH and CDGP. To summarize genotype–phenotype correlations, complete inactivation of GnRHR results in severe gonadotropic deficiency whereas partial gonadotropic deficiency results from partial GnRHR mutations, although these mutations do not preclude the occurrence of a severe phenotype in some patients.

The frequency of GnRHR loss of function mutations in nIHH is low. In a large retrospective analysis, we actually observed GnRHR mutations in 6% of patients with congenital isolated gonadotropic deficiency without anosmia (Leka and de Roux, unpublished results). This frequency increases up to 40% in familial cases with recessive mode of transmission, as reported by others (Beranova et al., 2001). Therefore, GnRHR mutations seem to be as frequent as TAC3 or TACR3 mutations in nIHH (Gianetti et al., 2010; Young et al., 2010).

6. Conclusion

Among all the genetic causes of isolated gonadotropic deficiency, GnRHR inactivating mutations were the first to be described. Since then, several groups have shown the difficulty to correlate the severity of the gonadotropic deficiency to the genotype. Functional analysis revealed that natural mutations of the GnRHR alter the GnRH-induced PLC signaling pathway by decreasing GnRH affinity, signal transduction or cell surface expression. Some of these mutations misfold the receptor and therefore block its exit from ER. Regarding all nIHH patients, the frequency of GnRHR mutations is relatively low, but explains almost 50% of familial cases with recessive transmission. In addition to GnRH/GnRHR, KiSS1R (GPR54) and TAC3/TACR3 inactivating mutations causing nIHH have now been described. However, few familial cases of nIHH remain unlinked to any of these five candidate genes.

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