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Mutations along the pituitary–gonadal axis affecting sexual maturation: Novel information from transgenic and knockout mice

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Abstract

During the last 10 years, numerous activating and inactivating mutations have been detected in the genes encoding the two gonadotrophins, luteinising hormone (LH) and follicle-stimulating hormone (FSH), as well as their cognate receptors (R), LHR and FSHR. Because activation of the hypothalamic–pituitary–gonadal axis is a crucial event in the onset and progression of puberty, mutations affecting gonadotrophin action have major influence on this developmental process. Many of the phenotypic effects observed have been expected on the basis of the existing information about gonadotrophin action (e.g. delayed puberty), but also many unexpected findings have been made, including the lack of phenotype in women with activating *LHR* mutations, and the discrepancy in phenotypes of men with inactivating mutations of *FSHβ* (azoospermia and infertility) and *FSHR* (oligozoospermia and subfertility). Some of the possible mutations, such as inactivating *LHβ* and activating *FSHR* mutations in women, have not yet been detected. Genetically modified mice provide relevant phenocopies for the human mutations and serve as good models for studies on molecular pathogenesis of these conditions. They may also predict phenotypes of the mutations that have not yet been detected in humans. We review here briefly the effects of gonadotrophin subunit and receptor mutations on puberty in humans and contrast the information with findings on genetically modified mice with similar mutations.

Keywords: Puberty; Gonadotrophins; Luteinising hormone (LH); Follicle-stimulating hormone (FSH); LH receptor (R); FSHR; Hypogonadism; Precocious puberty; Transgenic (TG) mice; Knockout (KO) mice; Mutation

1. Introduction

Nearly all possible types of mutations affecting the functions of gonadotrophins and their receptors are known today. They include inactivating mutations of the gonadotrophin subunits and inactivating and activating mutations of the gonadotrophin receptors. The only mutations not detected are inactivating mutations of the *glycoprotein hormone common α-subunit* (*Cα*) and *chorionic gonadotrophin β-subunit* (*hCGβ*), apparently because of the deleterious effect they would have on pregnancy. As expected, these mutations have marked effects on the timing and progression of puberty, manifesting both as its advancement and delay. Numerous genetically modified mouse models of gonadotrophin subunit and receptor mutations are also available today, and they provide in many cases accurate phenocopies of the respective human conditions. However, they have also revealed intriguing species differences in gonadotrophin action. Our purpose is, in this review, first to briefly summarise the current knowledge about the effects of human mutations of gonadotrophin subunits and receptors on puberty. We then review the currently available genetically modified mouse models for similar mutations, and how they have advanced our knowledge about the developmental aspects of gonadotrophin action. We concentrate in more detail on the two mouse models developed recently in our laboratory, the *LHR* knockout (KO) mouse (LuRKO) and the *hCG* overexpressing transgenic (TG) mouse (hCG+).

2. Influence of gonadotrophin and gonadotrophin receptor mutations on puberty in humans

The mutations in genes of hormones and their receptors can in principle be classified as activating and inactivating. Activating ligand mutations are extremely rare, and such alterations are not known in gonadotrophin subunit genes. All known gonadotrophin mutations are inactivating, however, there is a common polymorphisms in the *LHβ* gene (Trp8Arg/Ile15Thr) that increases the hormone's bioactivity *in vitro*, having thus

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some features of an activating mutation (Lamminen and Huhtaniemi, 2001). Even the inactivating gonadotrophin subunit mutations are very rare, but a number of activating and inactivating *LHR* and *FSHR* mutations have been discovered since 1993. More detailed reviews on this topic are available elsewhere (Huhtaniemi and Themmen, 2005; Themmen and Huhtaniemi, 2000).

2.1. FSHβ subunit and FSH receptor mutations

Several inactivating *FSHβ* mutations have been discovered in women with quite similar phenotype of primary amenorrhoea and infertility (Layman et al., 1997, 2002; Matthews et al., 1993). These patients have normal adrenarche but no signs of spontaneous gonadarche with primary amenorrhoea. Their basal oestradiol, progesterone and inhibin levels are low, LH high, and FSH is typically undetectable. Some subjects responds well to FSH treatment, which leads to activation of the ovarian function and even to successful pregnancy. A very similar phenotype is detected in cases with complete *FSHR* inactivation (Aittomaki et al., 1995). In addition to the phenotype of delayed puberty, the status of ovarian follicular development was studied in these patients, and in keeping with the knowledge about FSH action, they showed variable degree of developmental arrest (Aittomaki et al., 1996). Hence, both the FSH ligand and receptor mutations present with very similar phenotypes demonstrating that FSH action is crucial for the female pubertal development. Several partially inactivating *FSHR* mutations have also been described in women, and their phenotypes are, as expected, in principle similar but milder (Beau et al., 1998; Touraine et al., 1999).

Three men with inactivating *FSHβ* mutation have been described in the literature (Layman et al., 2002; Lindstedt et al., 1998; Phillip et al., 1998). Two of them had apparently normal puberty, which is expected because it is mainly induced by LH stimulated onset of testicular testosterone (T) production. Delayed puberty and hypogonadism, as evidenced by subnormal serum T, was detected in one of the men, but it is possible that his phenotype was not caused exclusively by the FSH deficiency. All three men were azoospermic, which is in contrast to the variable oligozoospermia and subfertility – but not azoospermia – that was detected in five men with homozygous inactivating *FSHR* mutation (Tapanainen et al., 1997). In principle, both inactivating FSH ligand and receptor mutations should produce similar phenotypes, as is the case with women. The reasons for this discrepancy in men remains open, and it may only be solved when the phenotypes of a larger number of affected individuals are known. Nevertheless, we can conclude from the available data that FSH inactivation has no major influence of the onset of pubertal maturation in men. With respect to the role of FSH in the onset of spermatogenesis, the available data on ligand and receptor mutations are controversial.

Only one apparently activating mutation of the *FSHR* has been described (Gromoll et al., 1996). The affected individual was a male who was previously hypophysectomised because of pituitary tumor, and despite unmeasurable gonadotrophin levels he had good spermatogenesis. A point mutation (Asp567Gly) was discovered in his *FSHR*, which displayed marginal constitutive activity upon *in vitro* testing. No women with activating *FSHR* mutations have yet been detected despite extensive search in candidate diseases and conditions, such as premature ovarian failure and multiple twin pregnancies. Very recently, different types of activating *FSHR* mutations were described in women, where the ligand specificity of the receptor is relaxed in such a manner that it becomes responsive to high concentrations of hCG, explaining the pathogenesis of pregnancy-associated ovarian hyperstimulation syndrome (Smits et al., 2003; Vasseur et al., 2003).

2.2. LHβ and LH receptor mutations

Only two men with documented inactivating *LHβ* mutation are known in the literature (Valdes-Socin et al., 2004; Weiss et al., 1992). Both were normally masculinised at birth but then presented with absence of spontaneous puberty and infertility. In one of the cases serum LH immunoreactivity was high, but it had no activity in radioreceptor assay, in the other there was no LH immunoreactivity because the mutated β-subunit was unable to dimerise with Cα. Testis biopsy was diagnostic in both cases, showing absent or hypoplastic Leydig cells and arrested spermatogenesis. These two cases demonstrate clearly that LH is not necessary for fetal masculinisation, because placental hCG can provide the stimulus for fetal Leydig cell androgen synthesis. However, pituitary LH is absolutely necessary for the pubertal onset of testicular endocrine function and development of the secondary sex characteristics. Curiously, no women with *LHβ* mutations have yet been detected. We can only guess that their phenotype would be the same as that of *LHR* inactivation (see below).

The first *LHR* mutations detected were activating, presenting with the dramatic phenotype of gonadotrophin independent male-limited precocious puberty (for a review, see Themmen and Huhtaniemi, 2000). Conspicuously, no phenotype has been described in female carriers of the same activating mutations, probably because *LHR* expression in the ovary needs FSH priming, which does not start until the onset of FSH secretion at the normal age of puberty. The next step was the discovery of inactivating *LHR* mutations, which, depending on the severity of inactivation, caused a hypogonadal phenotype in males, ranging from micropenis and hypospadias to complete pseudohermaphroditism (Themmen and Huhtaniemi, 2000). Interestingly, in this case, the receptor and ligand mutations differ; whereas complete *LHR* inactivation in male results in pseudohermaphroditism, the *LHβ* mutation allows normal fetal masculinisation and the lack of stimulation of testicular steroidogenesis becomes apparent only at puberty. The explanation is offered by hCG which can compensate for missing LH *in utero*, but cannot bypass the receptor inactivation. Inactivating mutations in women caused a milder phenotype of anovulatory infertility with largely normal pubertal maturation. Hence, FSH seems to be the more important gonadotrophin for female puberty and LH for male puberty.

In conclusion, we know now the phenotypes of inactivating *LHβ* mutations in men, but no females with such mutations have been identified. Likewise the phenotypes of activating *LHR*