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Molecular Genetics of the Follitropin Receptor: Structural Diversity, Mutations, Gene Knock Out and Biological Implications

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Pituitary follitropin (follicle stimulating hormone, FSH) an important member of the glycoprotein hormone family that is essential for reproduction functions by binding to a receptor(s) localized on discrete ovarian and testicular cells. The pre mRNA of the mammalian large single gene of >250kb (as deduced in the human genome) undergoes extensive alternative splicing creating proteins of different structural motifs. The mechanisms and relationships of four ovine follitropin receptor (FSH-R) variants that we have studied are highlighted in this article as example. These include the following – the classical Gs coupled receptor, a dominant negative form, a single transmembrane domain growth factor type I receptor and a potentially soluble form. As their signaling properties are different, it is proposed that receptor diversity exists to integrate hormone signaling mechanisms to coordinate developmental and steroidogenic functions of gonads. Several mutations of the follitropin receptor in humans observed in different parts of the world are known to compromise reproductive efficiency to varying degrees. Completely inactivating mutations are not common as the affected individual (particularly women) is rendered sterile. These conclusions are fully supported by studies in our receptor knockout mouse model (the FORKO mouse) in which we have deleted the entire FSH-R repertoire. The null females are sterile while the mutant males have reduced fertility. Mutant females as well as aging heterozygous females with estrogen deficiency and hormonal imbalances duplicate many conditions that exist in postmenopausal women. This model has provided unexpected experimental paradigms to investigate a variety of health issues related to quality of life during menopause. Thus, the null mutant and particularly the heterozygous female dubbed the "Menopause Mouse" exhibit osteoporosis, obesity, ovarian and uterine tumors, as well as changes in the brain regions associated with memory. Phenotypes in the +/- mice are age dependent. Mutant males also show distinct phenotypes with aberrant sperm and are also helpful in understanding the mechanisms that affect sperm production and quality. We propose that comparison of normal and mutant gonads and other steroid dependent target tissues will lead to the identification of downstream gene (products) involved in specific steps of their development and function. It is anticipated that the mutants might also be helpful in elucidating mechanisms related to ovarian and uterine pathogenesis.

Key Words : Aging, Andropause, Fertility, FSH receptor, Gene mutations, Gene knock out, Gene splicing, Menopause, Phenotypes, Polymorphism, Signalling

Introduction

The gonadotropins consisting of pituitary Follicle Stimulating Hormone (FSH, follitropin), Luteinizing Hormone (LH, lutropin) along with the placental gonadotropin (hCG-human choriogonadotropin) are among the best-characterized complex glycoprotein hormones regulating reproductive events.Pituitary thyrotropin (thyroid stimulating hormone-TSH) is also a related glycoprotein that controls thyroid functions. The two gonadotropins synthesized in the cells called pituitary gonadotropes are secreted as dimers under the influence of hypothalamic releasing factors that include the most well characterized gonadotropin releasing hormone (GnRH). The heterodimeric glycoprotein hormones

List of Abbreviations: *FSH*, Follicle Stimulating Hormone (Follitropin); *LH,* Luteinizing Hormone (Lutropin); *TSH*, Thyroid Stimulating Hormone (Thyrotropin); *hCG* human choriogonadotropin; *FSH-R,* Follitropin (FSH) receptor; *LH R*, Lutropin (LH) receptor; *GnRH*, Gonadotropin releasing hormone; *FORKO*, Follitropin receptor knock out.

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consist of a common α subunit non-covalently associated with a hormone specific β subunit that is structurally unique to each member of the family. Thus, in any given species (with some exceptions) the α subunits arise from a single gene whereas the β subunits originate from separate genes. Although pituitary lutropin and placental chorionic gonadotropin in primates [(urinary) hCG] are structurally and functionally homologous, the latter is a more potent hormone that is widely used in assisted reproductive treatments.

Follitropin is secreted as a highly heterogeneous hormone due to differences in post translational modifications such as glycosylation that could vary according to sex, age and cycle and for these reasons a variety of hormone isoforms are present in the pituitary and circulation at a given time (Ulloa-Aguirre et al. 1995). The hormone preparations used in clinical treatments (natural from urinary sources and more recent recombinant products) though highly purified are all a mixture of various isoforms. It is likely that the interaction of these follitropin isoforms with a given receptor repertoire present in the ovarian or testicular cell at any given stage will determine the final cellular response. The use of recombinant follitropin is gaining ground for both clinical and veterinary applications.

Follitropin binds to specific receptors that are localized predominantly in the gonads. In the female, hormone binding is localized in the ovarian follicle and expression is confined to the granulosa cells (Richards 1980). The acquisition of FSH receptor (FSH-R) is essential for granulosa cell differentiation, maturation and selection of the dominant follicle. Hormonal treatment causes up regulation of the receptor; however, the type and distribution of receptors may vary depending upon the developmental stage of the follicle and clearly demonstrated in an experimental model (Babu et al. 2001). Similarly in the male, FSH-R expression is also highly cell specific and confined to the nursing Sertoli cells that line the seminiferous epithelium in the testis (Kangasniemi et al. 1990). As in the ovary, FSH-R expression in the testis is also regulated according to the stages of spermatogenesis indicating coordinated and varied levels along the length of the seminiferous tubule (Heckert & Griswold 1993). Reports of the presence of FSH-R in germ cells in both sexes (see Simoni et al. 1997 for male, and Patsoula et al. (2001) Meduri et al. (2002) for oocytes and other sites in the reproductive tract (Kornyei et al. 1996, Mizrachi & Shemesh 1999) require a more thorough investigation. The high FSH-R expression levels occurring in granulosa cells that surround the developing oocyte (Babu et al. 2001) and the modulating effect of oocyte secreted factors emphasize the critical role of the hormone-receptor signaling system and bidirectional intercellular communication for successful gametogenesis and follicular development (Eppig et al. 2002).

This review, not meant to be exhaustive focuses on some recent studies on the molecular structure of the FSH-R gene, mutations, different mRNA transcripts, their signaling potential and regulation and an introduction to health related phenotypes in the knock out mouse model. The terms follitropin and FSH are used interchangeably in this article.

Molecular Genetics of the Follitropin Receptor

Following the first cloning of the rat RSH-R cDNA (Sprengel et al. 1990) the receptor from a number of other species including human (see Simoni et al. 1997) became available. Our laboratory that pursued the study of homologous hormone-receptor interactions in the testis cloned the classical Gs coupled form of the ovine FSH-R cDNA (Yarney et al. 1993) as well as a number of other structurally interesting variants (see below). The FSH-R like the LH-R and TSH-R belongs to a select class of G-protein coupled receptors which are characterized by the presence of seven transmembrane domains. The predicted structure of the hFSH-R consists of 695 amino acids that can be divided into 3 parts. A large hydrophilic extracellular domain of over 300 amino acids provides features for specific hormone recognition. The heptahelical structure that follows firmly anchors the receptor in the cell membrane. A third stretch of the protein consisting of the carboxyl terminal tail lies in the cytoplasm serving signaling functions along with several of the intracellular loops. This part of the protein

also contains a number of potential phosphorylation sites that could control receptor function as well as regulatory dynamics. The similarity in the organization of glycoprotein hormone receptors in man and other animals including distant species such as *Drosophila* has provided important information of evolutionary significance (Hauser et al. 1997).

Chromosomal Localization

In man FSH-R (as well as the LH-R) localization on chromosome 2 (2 p21) originally established by fluorescent in situ hybridization (see Simoni et al. 1997) is now confirmed by the recent human genome sequence. The mouse FSH-R lies on chromosome 17 according to the recently completed mouse genome sequence. In sheep and pig the receptor is localized on chromosome 3 (see Simoni et al. 1997). Unlike previous modest estimates of ~50-60 kb for the size gene, we can now infer from the human genome data that the structural gene is indeed very large spanning >250 kb on chromosome 2. Although it is currently believed that segments of the cDNA are encoded by 10 exons, with the 10th exon contributing the transmembrane domain and the cytoplasmic tail, emerging data from our laboratory is consistent with the genome structure suggesting that there is an 11th exon in the gene allowing the creation of unique spliced variants with structural motifs (figure 1&2) of potential physiological significance. This is an area that would require further study in many species including man.

The FSH-R Gene Promoter

The promoter is highly tissue and cell specific. For example with in the ovary it is expressed only in the granulosa cells at specific stages but not in the theca or interstitial cells of the ovary. Similarly in the testis it is expressed only in Sertoli cells but not Leydig cells that lie outside the seminiferous tubule. Therefore, there must be cis-elements in the promoter region that interact with unique trans-acting factors in the target cells to control FSH-R expression. To understand these regulations the promoter regions have been cloned from the human, ovine, rat and mouse for the purpose of mapping regulatory elements. In general these are large structures of several kilo bases containing both activating as well as repressive

Figure 1. Receptor diversity from a single gene. The single large >250 Kb ovine FSH receptor gene (based on analogy with the human genome) is shown to produce several major receptor forms. Four that have been well characterized in our laboratory are depicted in the diagram. FSH-R1- is the classical Gs coupled receptor; FSH-R2 –this is similar to R1 but only its carboxyl terminal 25 amino acids are different. FSH-R3- this is the growth factor type I receptor (see also figure 2) predicted to contain a single transmembrane segment. FSH-R4- is the potentially soluble receptor that has no transmembrane segment. In this receptor only the first four exons of FSH-R1 are retained. The number of amino acid residues for each mature protein is indicated. We believe there might be other forms yet to be discovered.

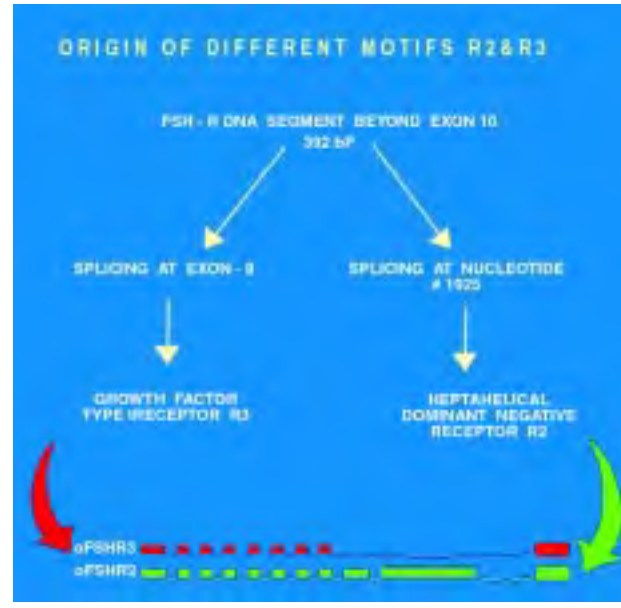

Figure 2. Use of same DNA segment to produce two different carboxyl termini in two receptor motifs. It is predicted that the oFSH-R3 transcript arises by splicing the 392 bp segment shown at the 8th exon creating a single transmembrane domain. When this same segment is spliced at nucleotide #1925 in exon 10 (same as R1) the dominant negative receptor R2 is produced. Its extreme carboxyl terminal is different from both R3 and R1.

DNA binding elements that could function in a stage specific manner (Heckert & Griswold 1993, Xing et al. 2002). Methylation of the promoter region could also play an important role in suppressing expression in non-gonadal cells (Heckert & Griswold 1993). Understanding these mechanisms and the promoter in man and other species could facilitate efforts to direct selective expression of genes to ovarian granulosa or testicular Sertoli cells for corrective therapy or transgenic studies.

Mutations of the FSH-R

Although the underlying reasons for infertility are complex, a knowledge of the receptor gene permits the examination of possible mutations in efforts to explain cases of infertility or other reproductive deficits in relation to clinical diagnosis. These could fall into several categories- outright germ line mutations of a homozygous nature that could inactivate the receptor, heterozygous mutations or polymorphisms either single or combined nature that could partially inactivate the gene or in some cases mutations that could lead to constitutive activation in absence of the ligand (i.e., follitropin). The first inactivating mutation of the FSH-R was described in 1995 in some Finnish family members with primary ovarian failure and this was originally characterized as pure gonadal dysgenesis (Aittomäki et al. 1995, Tapanainen et al. 1997, Jiang et al. 1998). This point mutation resulting in an Alanine 189 Valine amino acid substitution in the extracellular domain of the receptor led to almost complete receptor inactivation such that affected women could not sustain sufficient ovarian development. They were thus infertile. The same mutation affected male siblings to variable degrees. Although all these men showed clear signs of defective testicular function, spermatogenesis proceeded to some degree indicating (Tapanainen et al. 1997) that there are different thresholds required for FSH-R function in men and women. Clinical findings on families in Finland created a flurry of interest and it was originally thought this particular mutation could indeed be a cause of ovarian failure. As similar studies in other parts of the world could not find this mutation in infertile women or men, unknown modifier genes could be playing a role in particular populations. However, other sporadic or combined mutations contributing to primary or secondary amenorrhea have been reported (Beau et al. 1998, Touraine et al. 1999, Doherty et al. 2002). These also result in amino acid substitutions in the extracellular domain or elsewhere (Asp224Val/Leu601Val; Ala189Val/Ala419Thr; Ile160Thr/Arg573Cys). Another mutation Pro348Arg near the trans-membrane region caused primary amenorrhea and hypergonadotropic hypogonadism (Allen et al. 2003). The overall deleterious effect of FSH-R mutations on fertility particularly in women, explains why only few such cases are known. It is also possible that potential defects could lie elsewhere in the receptor gene; for example in the promoter region leading to inefficient receptor expression or other new exonic segments that are yet to be more thoroughly characterized. Thus, it is anticipated that continued interest in the molecular analysis of the FSH-R will bring us new data related to reproductive deficiencies in men and women.

Unlike the LH and TSH receptors where many constitutively activating mutations are known, only one such alteration (Asp567Gly) has been reported (Gromoll et al. 1996) in a hypophysectomized man who had normal spermatogenesis. Like the other two glycoprotein receptor this mutation is also in the transmembrane domain suggesting that activating mutations are generally localized in this segment of the receptor molecule.

Receptor Diversity-Molecular Basis and Functional Consequences

The phenomenon called alternative splicing of the pre mRNA plays a critical role in the production of diverse mRNA species from individual genes and this mechanism provides for an increase in the range of functional gene products in higher organisms. General estimates that there are apparently more than 100,000 proteins in a given cell and yet only about 30,000 functional genes are present in the 3 billion base pairs of the human genome appear to be discordant at a first glance. However, genome-wide analyses estimate that that alternative splicing occurs in at least 40-60% of human genes (Venter et al. 2001, Lander et al. 2001). These mechanisms combined with post translational modifications such as glycosylation and phosphorylation serve as useful molecular events devised by nature to

increase the diversity of protein structures. Thus a small number of genes have the vast potential to create nearly a million protein varieties in man and other complex living creatures to serve diverse regulatory functions.

G protein coupled receptors currently represent the largest target for the development of therapies for a wide variety of human diseases. In the G-protein coupled receptor family, diversity occurs either by the existence of several related individual genes (as is often the case) or by means of alternative splicing of single large genes. The latter mechanism prevails in the case of glycoprotein hormone receptors. Cloning of the FSH-R from different animal species first demonstrated the existence of several alternatively spliced mRNA transcripts in both the ovary and testis (Gromoll et al. 1992, 1993, Khan et al. 1993, Sairam et al. 1996, 1997, Yarney et al. 1997, Rajapaksha et al. 1996, Song et al. 2002). These studies reveal the high propensity of the large FSH-R gene to undergo alternative splicing giving rise to a number of different variants. Two basic arguments can be advanced in favor of these entities being physiologically significant. First, according to basic tenets of biochemistry, structural features are generally predictive of some biological function. Secondly, a common feature noted in all these variants of each species is the preservation of considerable stretches of the extracellular domain and confining the differences to the carboxyl terminus. This suggests that there could be opportunities for modulating signaling while maintaining some capacity to maintain hormone recognition. One can visualize that these variants could function individually or in concert with each other by homo or heterodimerization mechanisms.

Referring to the diagram in figure 1 that depicts four ovine receptor forms recognized in our studies as FSH-R1 to R4, several interesting features become apparent. The FSH-R1 and R2 structures are identical for almost the entire stretch except for the difference in the extreme carboxyl terminus. R3 is different from both R1 and R2 in that the molecule lacks the typical seven transmembrane domains. But by virtue of its single transmembrane topography it could be placed in the growth factor type I receptor family. Most interestingly even though R3 and R2 receptor types are different beyond exon 8 and the carboxyl terminal amino acid sequence, the DNA segment of the large gene that gives rise to these two variants are identical. Thus the scheme shown in figure 2 depicts the clever designs of nature, wherein the same (DNA) sequence from a putative exon 11 produces two variants depending upon the location of alternative splicing. Splicing occurring at nucleotide #1925 of exon 10 of R1 produces the R2 form in which the last 25 amino acids are different (from R1). When splicing of this same piece occurs after exon 8 of R1, a new structure with a single transmembrane structure R3 is created.

Having cloned several variants of the ovine FSH receptor cDNA in both the testis (Khan et al. 1993, Sairam et al. 1996, 1997, Yarney et al. 1997) and ovary (Babu et al. 1999, 2001) we have evaluated their potential signaling functions by expression in non-gonadal or gonadal cells that can be studied in culture. The FSH-R1 that is similar to the other classical G protein coupled receptors is effectively coupled to Gs causing activation of adenylate cyclase and enhancing cyclic A M P in the cell. The R2 that is also expressed on the cell surface in transfected cells is coupled

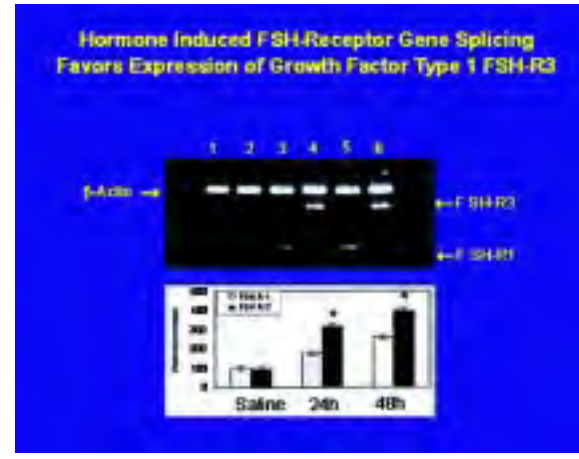

Figure 3. Hormonal regulation of FSH-R splicing. Data examining the expression of FSH-R1 and R3 using receptor specific primers for RT-PCR of ovaries from 21 day old immature female mice treated with a single injection of equine chorionic gonadotropin. Lanes 1 & 2 show saline treated mice; 3-4 are after 24 hrs of hormone and 5-6 show the effect after 48 hrs. The bottom panel shows a densitometric evaluation of expression normalized to β actin amplification in the same experiment. These mRNA changes of R3 are confirmed by Western blot analysis of protein levels in extracts using R3 specific anti-peptide receptor antibody. (figure based on Babu et al. 2001).

differently (presumably to Gi) inhibiting the production of cyclic AMP (Yarney et al. 1997). When R1 and R2 are co-expressed in cells, FSH induction of cyclic AMP is promptly abrogated suggesting a dominant negative effect (Sairam et al. 1996). In more recent studies, the functional significance of FSH-R3 has been explored both *in vitro* and *in vivo*. By comparing HEK 293 cells transfected with different receptors (Touyz et al. 2000), we have inferred that the cellular Ca^{2+} uptake functions of FSH are mediated by the R3 variant while the R1 or R2 are either weakly active or inactive. In additional studies we demonstrated that R3 but not R1 when expressed in immortal granulosa cells could cause the activation of map kinases ERK1 and ERK2 (Babu et al. 2000). The receptor gene splicing mechanism is under effective hormonal (follitropin itself) regulation because hormone priming regimens in the immature mouse that accelerate and synchronize follicular development favor the production of the FSH-R3 transcript as well as the corresponding protein (figure 3) (Babu et al. 2001). Although this regimen is strictly not identical to FSH priming protocols used in assisted reproductive treatments in the clinical settings, it is similar in principle. For this reason the observed regulation of FSH-R gene splicing to coordinate cellular events in the follicle could assume significance in determining follicular selection and dominance. Considering the fact that FSH has mitogenic as well as steroidogenic effects on granulosa cells and both the FSH-R1 and R3 are able to exert these effects to differing degrees (Babu et al. 2001), we postulate that a balance in the expression and actions of these two receptors could tilt the behavior of the cell towards one or the other pathways (see figure 4). Similarly, differences noted in FSH responses and receptor signaling along the length of the seminiferous tubule that seem to vary according to the stages of spermatogenesis (Kangasniemi et al. 1990, Heckert & Griswold 1993, Simoni et al. 1997) could also be due to the opposing interactions of receptor variants.

A recipe for fine tuning: As mentioned earlier, the hormone follitropin itself is produced as different forms in the pituitary and exists in circulation in polymorphic forms due to differences in glycosylation. In addition to influencing clearance patterns, recent evidence

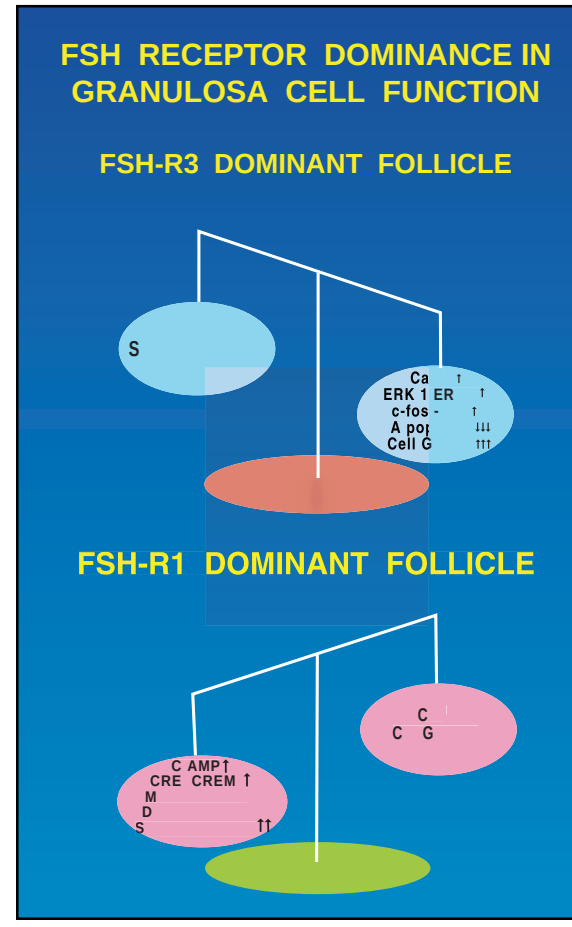

Figure 4. Balanced regulation of FSH-R3 and R1 during ovarian follicle development and function. Based on results shown in figure 3 and their signaling functions, we postulate that receptor related dynamic events. When early and rapid growth is required R3 action dominates; when a robust steroidogenic function becomes necessary in a more differentiated state R1 action prevails.

emerging from studies examining the actions of separated hormone isoforms (Vitt et al. 1998, 2001) suggests they might have different stimulating potencies at the cellular level. In view of the potential for generating cell stage dependent spliced receptor variants as discussed above, one can visualize intricate mechanisms at play in which both hormone and receptor variants interact in a coordinated manner creating a recipe for fine tuning ovarian and testicular responses. Understanding these intricate mechanisms will be a challenge for the future.

In this context we should also consider the interesting possibility that single nucleotide polymorphisms of the FSH-R could be among the factors determining the overall response of the

ovary or testis in individuals undergoing clinical treatment. Thus, some recent studies have begun reporting that different amounts of FSH are required for treating individuals with discrete receptor variants Thr307Asn680 and Ala307/Ser680 (see Perez et al. 2000, Sudo et al. 2003). Thus in the future, FSH-R genotyping might provide a helpful hint for initiating tailored treatment protocols balancing adequate stimulation vs. hyperstimulation.

The FORKO Mouse and Phenotypes

The application of molecular biological and genetic techniques have permitted the generation of numerous animal transgenic models either by over expressing a particular gene at a desired cellular location or its deletion by homologous recombination methods. In the latter instance a so called gene knock out is generated of which there can be two types. The first in which the gene is deleted from the germ line and second in which a conditional knock out can be induced whenever desired during post natal or adult life. If the gene is expressed in multiple locations a conditional knockout serves to inactivate the gene in a selected location of interest. Furthermore, it is also possible to achieve deletion at any given period of life e.g.; at a specified age. Currently these genetic manipulations, particularly the knock outs are easily performed in the laboratory mouse and the effects are examined in strains of different genetic background to understand the potential effect of modifier genes on the phenotypic outcome.

Fortunately as the FSH-R gene is confined to a predominantly select cell type in the ovary and testis, a knock out strategy deleting the expression of the entire repertoire is feasible and permits a study of the biological consequences. Accordingly we produced the first knockout model of a glycoprotein hormone receptor by deleting the FSH-R in the mouse (Dierich et al. 1998). We have subsequently called this model the FORKO (**F**ollitropin **R**eceptor **K**nock **O**ut) mouse (Krishnamurthy et al. 2000, Danilovich et al. 2000). This designation implies that the animals lack all FSH-R's in the null (-/-) state. The heterozygous mice [haploinsufficient (+/-) state] express only 50% of the gene. Subsequent to our report another group (Abel et al. 2000) also obtained a knock out of the FSH-R and essentially confirmed the changes in fertility status. The FORKO phenotypes which are numerous to describe in detail here are very interesting and informative. These depict the role of the FSH-R in reproductive events in both the female and male as well as other general health/aging related issues. From a systematic follow up of these mutants the following main conclusions can be drawn by classifying the phenotypes in to two broad categories (see figure 5). First, the phenotypes in females are dramatic being genotype and age dependent. Null females are sterile with atrophic reproductive organs and failure of follicular development beyond the late secondary/preantral stages. Our recent findings (unpublished data) challenge existing concepts of gonadotropin independence of early follicular development in rodents. Although some follicles may proceed to

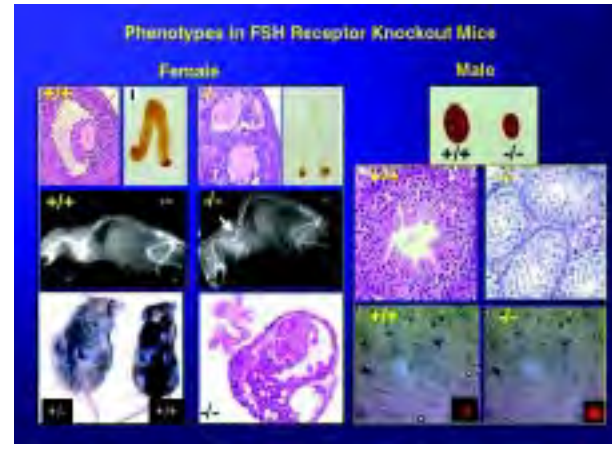

Figure 5. Examples of phenotypes in follitropin receptor knockout (FORKO) mice: Both male and female phenotypes are shown for animals at 3 months of age except otherwise indicated. Female mice: Clockwise-Top left show ovarian follicle and uterus in +/+ mouse. Right –Null ovary and uterus. Note small ovary and atrophied thread like uterus. Middle panel-Whole body x-ray of +/+ mouse. Right Null mouse showing curved vertebral column (kyphosis). Bones are fragile. Bottom-Left compares the body size of +/- (obese due to excessive adipose tissue) and wild type mice with normal FSH-R function. Right –Example of late stage ovarian tumor in a 1 yr old null mouse.

Right panels show changes in males. Top panels compare wild type and FORKO testis that are about half the normal size. Middle compares the tubular structures. Left shows a +/+ section with normal spermatogenesis. Right depicts shrunken tubules and impaired spermatogenesis in -/- testis. Bottom left is normal sperm of +/+ with head stained with propidium iodide at the corner. Right- in -/- males most sperm are abnormal with retention of cytoplasmic droplets in the tail. Note enlarged head in -/- sperm at the corner suggestive of improper chromatin condensation.

limited stages of development, the overall dynamics are affected to such degree which indicates that FSH-R signaling plays a decisive role in modulating this process. Thus gonadotropin dependence at an early stage need not be visualized as being an all or none event. As there is complete failure of ovulation in the null FORKO, no corpora lutea are present and loss of fertility is irreversible. The profound hormonal imbalances created in the mutants, most particularly estrogen deficiency and elevated testosterone, produce a variety of phenotypes that are reminiscent of the menopausal state or other aberrant conditions in women. As early as 3 months of age the null mice experience obesity (excess abdominal fat), skeletal abnormality (osteoporosis) (Danilovich et al. 2000) and ovarian tumors associated with aging (Danilovich et al. 2001). Most of these phenotypes are also seen in +/- females although they become perceptible at a later age. Similar to premature ovarian failure seen in some women, the +/- female mice that have reduced fertility in the beginning experience early loss of oocytes and become infertile after few rounds of breeding. This change in reproductive performance highlights the importance of the FSH-R in maintaining ovarian reserves (Danilovich & Sairam 2001). These +/- FORKO females also develop uterine abnormalities that afflict many middle aged women (Danilovich et al. 2002). For a variety of these reasons, the +/- FORKO animal in popular science terms has been dubbed the "Menopause mouse" (Beckman 2002). We predict that virtually all estrogen dependent functions including memory deficits (Tam et al. 2002) and androgen excess associated pathologies will be eventually discovered in these animals. As the estrogen receptors are intact in the target tissues of these mice (Danilovich et al. 2000, Sairam et al. 2002) they would be useful in development of site selective estrogen and/ or androgen receptor modulators for treatment.

Interesting phenotypes also become apparent in the mutant males although the severity is not as dramatic as in the females. The testis in FORKO males remains underdeveloped from the very young age (Dierich et al. 1998, Krishnamurthy et al. 2000, Krishnamurthy et al. 2001) and never recovering their normalcy despite the presence of high circulating FSH levels. This rules out any possibility of transactivation via other related receptors. Although young animals have normal testosterone, adults start experiencing lower androgen levels with this deficiency persisting in to aging animals indicating perturbation of intercellular communication between the Sertoli and Leydig cells (Krishnamurthy et al. 2001). As we are beginning to recognize metabolic changes typical of androgen deficiency (unpublished data) the null males could become an experimental model for studying andropause in men. Puberty is delayed in FORKO males and sperm production and quality are clearly compromised. The mutant males exhibit reduced fertility due to defects in their sperm. The major sperm abnormalities include retention of cytoplasmic droplets; decrease in motility and viability, and inefficient chromatin condensation. These are among several signs that are also associated with infertility in some men. The combination of these abnormalities reduces fertility in the FORKO males; although some mutants remain infertile. As mentioned previously, some of the Finnish men homozygous for an inactivating mutation of the FSH-R (Tapanainen et al. 1997) are also infertile indicating that the threshold for the obligatory nature of receptor function is variable among males and is also different from women where hormone dependence is critical. While some investigators might be inclined to regard the reduced fertility of FORKO males as an indication that FSH/FSH-R signaling is dispensable for spermatogenesis, we tend to take a practical view that this system is essential for maintaining normal fertility. Nature would not design and sustain such an important hormone-receptor system through evolution without the need to perform a critical function in the testis.

In conclusion, recent investigations have shown the diversity in variants of the FSH-R that arise from alternative splicing of the single gene and the possibility that they might be connected to different signaling pathways. The occurrence of variants in differing ratios could be part of a physiological mechanism for regulating gonadal response to the incoming hormone that in itself is also polymorphic in nature. Several mutations of the FSH-R that might contribute to reproductive deficits in humans have been recognized with

scope for additional discoveries. The knockout mouse model of the FSH-R confirms its essential nature for sustaining gonadal functions opening opportunities for investigating many hormone related health issues in women and men.

Prospects for Future studies:

The last fifteen years have produced a wealth of knowledge on the molecular biology, genetics, physiology and cellular mode of action of follitropin and its receptors. Advances in both fundamental science and clinical fronts have been impressive with the use of recombinant hormones gaining popularity. With the knowledge of the human and mouse genomes on hand we can expect more intensive explorations of mutations as related to pathology, deficiency states and impact of genotypes on treatments. Further studies on splicing patterns, mechanisms of signaling, identification of protein interacting partners, actions of hormonal isoforms, relevance of receptor expressions in other cells and gene/proteomic analysis in mutants are necessary. As there could be species variations in hormone-receptor interactions, valuable lessons could be forth coming from studies on different models.

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References

Abel M H, Wootton A N, Wilkins V, Huhtaniemi I, Knight P G, Charlton H M 2000 The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction; *Endocrinology* **141** 1795-803

Aittomäki K, Dieguez Lucena J L, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila E M, Lehväslaiho H, Engel AR, Nieschlag E, Huhtaniemi I and de la Chapelle A 1995 Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure; *Cell* **82** 959-968

Allen L A, Achermann J C, Pakarinen P, Kotlar T J, Huhtaniemi I T, Jameson J L, Cheetham T D and Ball S G 2003 A novel loss of function mutation in exon 10 of the FSH receptor gene causing hypergonadotrophic hypogonadism: clinical and molecular characteristics; *Hum. Reprod.* **18** 251-256

Babu P S, Danilovich N and Sairam M R 2001 Hormone-induced receptor gene splicing: enhanced expression of the growth factor type I follicle-stimulating hormone receptor motif in the developing mouse ovary as a new paradigm in growth regulation; *Endocrinology* **142** 381-389

_____, Jiang L, Sairam A M, Touyz R M and Sairam M R 1999 Structural features and expression of an alternatively spliced growth factor type I receptor for follitropin signaling in the developing ovary; *Mol. Cell. Biol. Res. Commun.* **2** 21-27

_____, Krishnamurthy H, Chedrese P J and Sairam M R 2001 Activation of extracellular regulated kinase pathways in ovarian granulosa cells by the novel growth factor type I follicle-stimulating hormone receptor: role in hormone signaling and cell proliferation; *J. Biol. Chem.* **275** 27615-27626

Beau I, Touraine P, Meduri G, Gougeon A, Desroches A, Matuchansky C, Milgrom E, Kuttenn F and Misrahi M 1998 A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor; *J. Clin. Invest.* **102** 1352-1359

Beckman M 2002 Menopause Mouse. Hormone-hampered rodents mimic menopause. Science's SAGE KE (14 August) **32** 112

Danilovich N and Sairam M R 2001 Haploinsufficiency of the follicle-stimulating hormone receptor accelerates oocyte loss inducing early reproductive senescence and biological aging in mice; *Biol. Reprod.* **67** 361-369

_____, Babu P S, Xing W, Gerdes M, Krishnamurthy H and Sairam M R 2000 Estrogen deficiency, obesity, and skeletal abnormalities in follicle-stimulating hormone receptor knockout (FORKO) female mice; *Endocrinology* **141** 4295-4308

_____, Roy I and Sairam M R 2001 Ovarian pathology and high incidence of sex cord tumors in follitropin receptor knockout (FORKO) mice; *Endocrinology* **142** 3673-3684

Danilovich N, Roy I and Sairam M R 2002 Emergence of uterine pathology during accelerated biological aging in FSH receptor haploinsufficient mice; *Endocrinology* **143** 3618-3627

Dierich A, Sairam M R, Monaco L, Fimia G M, Gansmuller A, LeMeur M and Sassone-Corsi P 1998 Impairing follicle-stimulating hormone (FSH) signaling *in vivo*. Targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance; *Proc. Natl. Acad. Sci. (USA)* **95** 13612-13617

Doherty E, Pakarinen P, Tiitinen A, Kiilavuori A, Huhtaniemi I, Forrest S and Aittomaki K 2002 A Novel mutation in the FSH receptor inhibiting signal transduction and causing primary ovarian failure; *J. Clin. Endocrinol. Metab.* **87** 1151-1155

Eppig J J, Wigglesworth K and Pendola F L 2002 The mammalian oocyte orchestrates the rate of ovarian follicular development; *Proc. Natl. Acad. Sci. U S A* **99** 2890-2894

Gromoll J, Dankbar B, Sharma R S and Nieschlag E 1993 Molecular cloning of the testicular follicle stimulating hormone receptor of the non human primate Macaca fascicularis and identification of multiple transcripts in the testis; *Biochem. Biophys. Res. Commun.* **196** 1066-1072

_____, Gudermann T and Nieschlag E 1992 Molecular cloning of a truncated isoform of the human follicle stimulating hormone receptor; *Biochem. Biophys. Res. Commun.* **188** 1077-1083

_____, Simoni M and Nieschlag E 1996 An activating mutation of the follicle stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man; *J. Clin. Endocrinol. Metab.* **81** 1367-1370

Hauser F, Nothacker H P and Grimmelikhuijzen C J P 1997 Molecular cloning, genomic organization, and developmental regulation of a novel receptor from *Drosophila melanogaster* structurally related to members of the thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone/ choriogonadotropin receptor family from mammals; *J. Biol. Chem.* **272** 1002-1010

Heckert L L and Griswold M D 1993 Expression of the FSH receptor in the testis; *Recent. Prog. Horm. Res.* **48** 61-77

Jiang M, Aittomaki K, Nilsson C, Pakarinen P, Tiitia A, Torresani T, Simonsen H, Goh V, Pettersson K, de la Chapelle A and Huhtaniemi I 1998 The frequency of an inactivating point mutation (566C–>T) of the human follicle-stimulating hormone receptor gene in four populations using allele-specific hybridization and time-resolved fluorometry; *J. Clin. Endocrinol. Metab.* **83** 4338-4343

Kangasniemi M, Kaipia A, Toppari J, Perheentupa A, Huhtaniemi I and Parvinen M 1990 Cellular regulation of follicle-stimulating hormone (FSH) binding in rat seminiferous tubules; *J. Androl.* **11** 336-43

Khan H, Yarney T A and Sairam M R 1993 Cloning of alternative spliced mRNA transcripts coding for variants of ovine testicular follitropin receptor lacking the G protein coupling domains; *Biochem. Biophys. Res. Commun.* **190** 888-894

Kornyei J L, Li X, Lei Z M, Rao C V 1996 Restoration of human chorionic gonadotropin response in human myometrial smooth muscle cells by treatment with follicle-stimulating hormone (FSH): evidence for the presence of FSH receptors in human myometrium; *Eur. J. Endocrinol.* **134** 225-231

Krishnamurthy H, Danilovich N, Morales C and Sairam M R 2000 Qualitative and quantitative decline in spermatogenesis of the follicle stimulating hormone receptor knock-out (FORKO) mouse; *Biol. Reprod.* **62** 1146-1159

_____, Kats R, Danilovich N, Javeshghani D and Sairam M R 2001 Intercellular communication between Sertoli cells and Leydig cells in the absence of follicle-stimulating hormone receptor signaling; *Biol. Reprod.* **65** 1201-1207

_____, Suresh Babu P, Morales C R and Sairam M R 2001 Delay in sexual maturity of the follicle stimulating hormone receptor knockout (FORKO) male mouse; *Biol. Reprod.* **65** 522-531

Lander E S et al. 2001 International Human Genome Sequencing Consortium; *Nature* **409** 860-921

Meduri G, Charnaux N, Driancourt M A, Combettes L, Granet P, Vannier B, Loosfelt H and Milgrom E 2002 Follicle-stimulating hormone receptors in oocytes; *J. Clin. Endocrinol. Metab.* **87** 2266-2276

Mizrachi D and Shemesh M 1999 Follicle-stimulating hormone receptor and its messenger ribonucleic acid are present in the bovine cervix and can regulate cervical prostanoid synthesis; *Biol. Reprod.* **61** 776-84

Patsoula E, Loutradis D, Drakakis P, Kallianidis K, Bletsa R, Michalas S 2001 Expression of mRNA for the LH and FSH receptors in mouse oocytes and preimplantation embryos; *Reproduction.* **121** 455-461

Perez Mayorga M, Gromoll J, Behre H M, Gassner C, Nieschlag E and Simoni M 2000 Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype; *J. Clin. Endocrinol. Metab.* **85** 3365-3369

Rajapaksha W R, Robertson L and O'Shaughnessy P J 1996 Expression of follicle-stimulating hormone-receptor mRNA alternate transcripts in bovine granulosa cells during luteinization in vivo and in vitro; *Mol. Cell. Endocrinol.* **120** 25-30

Richards J S 1980 Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation; *Physiol. Rev.* **60** 51-89

Sairam M R, Danilovich N and Lussier-Cacan S 2002 The FORKO mouse as a genetic model for exploring estrogen replacement therapy; *J. Reprod. Med.* **47** 412-418

_____, Jiang L G, Khan H and Yarney T A 1996 Follitropin signal transduction: Alternative splicing of the FSH receptor gene produces a dominant negative form of receptor which inhibits hormone action; *Biochem. Biophys. Res. Commun.* **226** 717-722

_____, _____, Yarney T A and Khan H 1997 Alternative splicing converts the G-protein coupled follitropin receptor gene into a growth factor type I receptor: Implications for pleiotropic actions of the hormone; *Mol. Reprod. Develop.* **48** 471-479

Simoni M, Gromoll J and Nieschlag E 1997 The follicle stimulating hormone receptor. Biochemistry, molecular biology, physiology and pathophysiology; *Endocr. Rev.* **18** 739-773

Song G J, Park Y S, Lee Y S, Lee C C and Kang I S 2002. Alternatively spliced variants of the follicle-stimulating hormone receptor gene in the testis of infertile men; *Fertil. Steril.* **77** 499-504

Sprengel R, Braun T, Nikolics K, Segaloff D L and Seeburg P H 1990 The testicular receptor for follicle stimulating hormone: structure and functional expression of cloned cDNA; *Mol. Endocrinol.* **4** 525-530

Sudo S, Kudo M, Wada S, Sato O, Hsueh A J W and Fujimoto S 2003 Genetic and functional analyses of polymorphisms in the human FSH receptor gene; *Mol. Hum. Reprod.* **8** 893-899

Tam J, Danilovich N, Nilsson K, Sairam M R and Maysinger D 2002 Chronic estrogen deficiency leads to molecular aberrations related to neurodegenerative changes in follitropin receptor knockout female mice; *Neuroscience* **114** 493-506

Tapanainen J S, Aittomaki K, Min J, Vaskivuo T and Huhtaniemi I T 1997 Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility; *Nat. Genet.* **15** 205-206

Touraine P, Beau I, Gougeon A, Meduri G, Desroches A, Pichard C, Detoeuf M, Paniel B, Prieur M, Zorn J R, Milgrom E, Kuttenn F and Misrahi M 1999 New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype; *Mol. Endocrinol.* **13** 1844-1854

Touyz R M, Jiang L and Sairam M R 2000 Follicle stimulating hormone mediated calcium signaling by the alternatively spliced growth factor type I receptor; *Biol. Reprod.* **62** 1067-1074

Ulloa-Aguirre A, Midgley A R Jr, Beitins I Z and Padmanabhan V 1995 Follicle-stimulating isohormones: characterization and physiological relevance; *Endocr. Rev.* **16** 765-787

Venter J C, et al. 2001 The sequence of the human genome; *Science* **291** 1304-1351

Vitt U A, Kloosterboer H J, Rose U M, Mulders J W, Kiesel P S, Bete S and Nayudu P L 1998 Isoforms of human recombinant follicle-stimulating hormone: comparison of effects on murine follicle development in vitro; *Biol. Reprod.* **59** 854-861

_____, Nayudu P L, Rose U M and Kloosterboer H J 2001 Embryonic development after follicle culture is influenced by follicle-stimulating hormone isoelectric point range; *Biol. Reprod.* **65** 1542-1547

Xing W, Danilovich N and Sairam M R 2002 Orphan receptor chicken ovalbumin upstream promoter transcription factors inhibit steroid factor-1, upstream stimulatory factor, and activator protein-1 activation of ovine follicle-stimulating hormone receptor expression via composite cis-elements; *Biol. Reprod.* **66** 1656-1666

Yarney T A, Jiang L G, Khan H, MacDonald E A, Laird D W and Sairam M R 1997 Identification of an isoform of the ovine testicular FSH receptor bearing a variant carboxyl terminus: Structure and functional expression; *Mol. Reprod. Develop.* **48** 458-470

Yarney T A, Sairam M R, Khan H, Ravindranath N, Payne S and Seidah N G 1993 Molecular cloning and expression of the ovine testicular follicle-stimulating hormone receptor; *Mol. Cell Endocrinol.* **93** 219-226