

New insights in prolactin: pathological implications

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Abstract | Prolactin is a hormone that is mainly secreted by lactotroph cells of the anterior pituitary gland, and is involved in many biological processes including lactation and reproduction. Animal models have provided insights into the biology of prolactin proteins and offer compelling evidence that the different prolactin isoforms each have independent biological functions. The major isoform, 23 kDa prolactin, acts via its membrane receptor, the prolactin receptor (PRL-R), which is a member of the haematopoietic cytokine superfamily and for which the mechanism of activation has been deciphered. The 16 kDa prolactin isoform is a cleavage product derived from native prolactin, which has received particular attention as a result of its newly described inhibitory effects on angiogenesis and tumorigenesis. The discovery of multiple extrapituitary sites of prolactin secretion also increases the range of known functions of this hormone. This Review summarizes current knowledge of the biology of prolactin and its receptor, as well as its physiological and pathological roles. We focus on the role of prolactin in human pathophysiology, particularly the discovery of the mechanism underlying infertility associated with hyperprolactinaemia and the identification of the first mutation in human *PRLR*.

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Introduction

First discovered nearly 90 years ago, prolactin stimulates the proliferation and differentiation of the mammary cells required for lactation. In humans, the principal prolactin-related symptoms, such as hypogonadism and infertility, result from hypersecretion of this hormone. The prolactin receptor (PRL-R) is ubiquitously expressed but tissue-specific variability in expression patterns also exist.^{1,2} Studies of animal models have assigned multiple functions to prolactin, both physiological (reproduction, lactation, growth, metabolism, electrolyte transport and behaviour) and pathological (immunity and carcinogenesis).^{3–5} Over the past decade, many new insights into the functions of prolactin and its receptor have been revealed, including identification of the first inactivating mutation in the human gene encoding PRL-R, *PRLR*, and the mechanism by which high levels of prolactin can lead to gonadotropin deficiency and infertility, as well as the role of the 16 kDa proteolytic fragment of prolactin in regulation of angiogenesis.⁶ This Review focuses on the novel findings that concern the role of prolactin in human pathophysiology.

The prolactin gene and protein variants Structure and post-translational modifications

Prolactin is encoded by a single gene, *PRL*, that is conserved among all vertebrates and is located on chromosome 6 in humans.⁷ Although it was initially thought that the *PRL* gene consisted of five exons and four introns,⁷ an additional noncoding exon, 1a, has also been described.⁸

Following cleavage of the 28 amino acid signal peptide, the mature 23 kDa protein consists of 199 amino acids.⁹ Prolactin has strong structural homology with growth hormone and placental lactogen and belongs to a large haematopoietic cytokine family of proteins characterized by a 3D structure comprising four antiparallel α helices.¹⁰ Numerous variants of the prolactin protein have been identified in plasma and the pituitary gland, many of which result from post-translational modifications of the mature protein, including phosphorylation,¹¹ glycosylation, sulphation and deamidation.⁹ In addition to monomeric 23 kDa prolactin, two other major forms of the protein are present in the circulation. Referred to as 'big PRL' and 'big big PRL' (also known as macroprolactin), these high molecular mass (>100 kDa) complexes of 23 kDa prolactin and IgG autoantibodies can be detected to varying degrees by prolactin immunoassays;¹² however these forms of prolactin have minimal biological activity *in vivo* and no known pathological functions.¹² Macroprolactinaemia is also termed analytical hyperprolactinaemia, and its presence in the sera of patients can lead to clinical dilemmas due to the potential misinterpretations of biochemical testing. Current best practice recommends that serum is subfractionated using polyethylene glycol precipitation to provide increased quality of the measurement of bioactive monomeric prolactin.^{13–15}

The 14 kDa, 16 kDa and 22 kDa prolactin variants are generated from proteolytic cleavage of the 23 kDa protein.⁹ The 16 kDa variant is a product of cleavage of prolactin at the long loop that connects the third and fourth α helices by cathepsin D or matrix metalloproteases.⁶ This cleavage can occur outside the cells in the interstitial medium

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Competing interests

The authors declare no competing interests.

Key points

- The major 23 kDa prolactin isoform exerts its action via a transmembrane receptor, prolactin receptor (PRL-R), which belongs to the class of haematopoietic cytokine receptors
- Binding of prolactin to its predimerized receptor induces a conformational change in the receptor, which enables signal transduction
- Hyperprolactinaemia causes hypogonadotropic hypogonadism by inhibiting kisspeptin-1 secretion, which in turn disrupts hypothalamic gonadotropin-releasing hormone I secretion
- The first germline loss-of-function mutation in the gene that encodes PRL-R was reported in three sisters with familial idiopathic hyperprolactinaemia
- The 16 kDa isoform of prolactin has antitumoral and antiangiogenic actions and is involved in peripartum cardiomyopathy

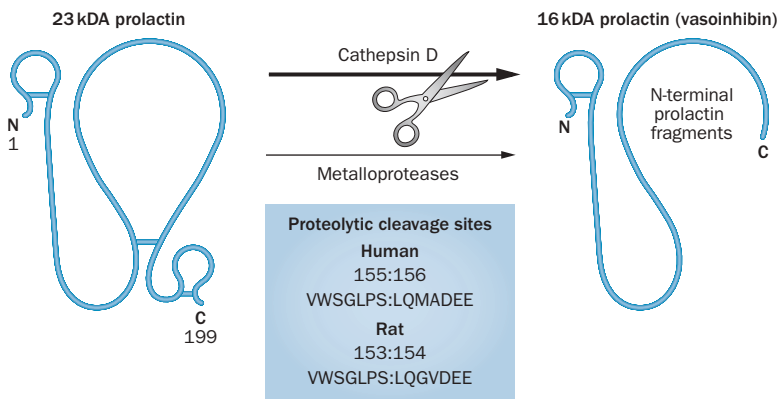


Figure 1 | Prolactin isoforms. Human 23 kDa prolactin (1–199 amino acids) is cleaved by cathepsin D or metalloproteases between Ser155 and Leu156 to yield the antiangiogenic N-terminal 16 kDa prolactin. This cleavage can occur outside the cells in the vicinity of blood capillaries. Prolactin amino acid sequences neighbouring the cleavage site in the human (Ser155–Leu156) and the rat (Ser153–Leu154) hormones are also shown.

and, therefore, in the vicinity of blood capillaries, which implies that tissue-specific mechanisms of regulation exist. Importantly, the 16 kDa prolactin variant contains only the N-terminal part of the mature protein and, thus, has lost the ability to bind PRL-R (Figure 1).^{16,17} New evidence suggests that this variant is produced in several tissues, such as the retina,¹⁸ myocardium,¹⁹ chondrocytes¹⁷ and mammary gland.²⁰ In addition, the 16 kDa variant seems to be able to bind to endothelial cells¹⁶ and has inherent antiangiogenic properties,²¹ which has led to use of the term ‘vasoinhibin’ for this protein.⁶

Tissue-specific expression

Prolactin is mainly synthesized and secreted by lactotroph cells of the anterior pituitary gland, and this process is under the control of multiple stimulatory and inhibitory factors. Dopamine, which is secreted by tuberoinfundibular hypothalamic neurons, is the primary inhibitory regulator of secretion of pituitary prolactin (pPRL);²² this inhibitory tone is exerted via D₂ dopamine receptors on the surface of lactotroph cells. pPRL secretion can be enhanced by factors such as TSH-releasing factor and estrogens. pPRL exerts a negative feedback effect on its own secretion, mainly in an indirect manner by stimulating hypothalamic dopamine secretion, but possibly also through a direct action on lactotroph cells.²³

Studies have also demonstrated extrapituitary prolactin (ePRL) synthesis and secretion in several tissues and peripheral organs, which is regulated by mechanisms different from those that regulate pPRL.²⁴ Among the differences between pPRL and ePRL, ePRL mRNA contains an additional 150 bp, owing to extrapituitary transcription of the noncoding exon 1a under the control of a distal promoter.²⁵ By contrast, transcription of pPRL is regulated by a proximal promoter, which is also called the pituitary promoter.²⁶ The protein structures of pPRL and ePRL are identical and both forms bind to PRL-R. The main sites of ePRL synthesis and secretion are the decidua, mammary gland, ovary, prostate, testis, lymphocytes, endothelial cells and brain;²⁷ other identified sources are the skin and hair follicles,²⁸ adipose tissue²⁹ and cochlea.³⁰ The regulation and function of ePRL within these organs has been reviewed extensively elsewhere.²⁴ Nevertheless, the existence of ePRL seems to be more frequent in humans than in other animals.²⁷ Apart from its autocrine functions in mammary epithelial cells where it serves to induce terminal differentiation during late pregnancy,³¹ little is known about the physiological role of locally produced ePRL compared with the pleiotropic actions usually attributed to endocrine pPRL.³ Despite efforts to measure the levels of ePRL, it remains difficult to attach importance to its role in the tissues in which it has been detected.

PRL-R

Isoforms and structure

The actions of prolactin are mediated by its transmembrane receptor, PRL-R, which is a member of the haematopoietic cytokine receptor superfamily.³ The structure of members of this receptor family is unique and comprises an extracellular domain with two disulphide bridges that are essential for ligand binding, as well as a duplicated tryptophan–serine (WS) motif,³² a single transmembrane domain and an intracellular signal-transducing domain (Figure 2). The cytoplasmic domain contains two regions (Box 1 and Box 2) that are highly conserved among cytokine receptors.³³ Box 1 is a membrane-proximal region composed of eight amino acids, is very rich in proline and hydrophobic residues and adopts a consensus folding conformation that is specifically recognized by transducing tyrosine kinases.^{34,35} The second consensus region, Box 2, is much less conserved than Box 1 and consists of a succession of hydrophobic negatively charged residues followed by positively charged residues.³⁶

The PRLR gene is unique in all species (located on chromosome 5 in humans and chromosome 15 in mice).^{37–39} In mammals, PRLR contains at least 10 exons, but alternative splicing results in the generation of several different isoforms (Figure 2). These isoforms have an identical extracellular domain, but differ in the size and sequence of the intracellular portion, which can be short, intermediate or long.^{3,40–42} Short forms of the receptor lack the cytoplasmic domain. In addition to the different membrane-bound isoforms, a soluble PRL-R has been identified (termed PRL-binding protein), which contains only the extracellular domain of the membrane receptor. Depending on the species, PRL-binding protein can be generated from

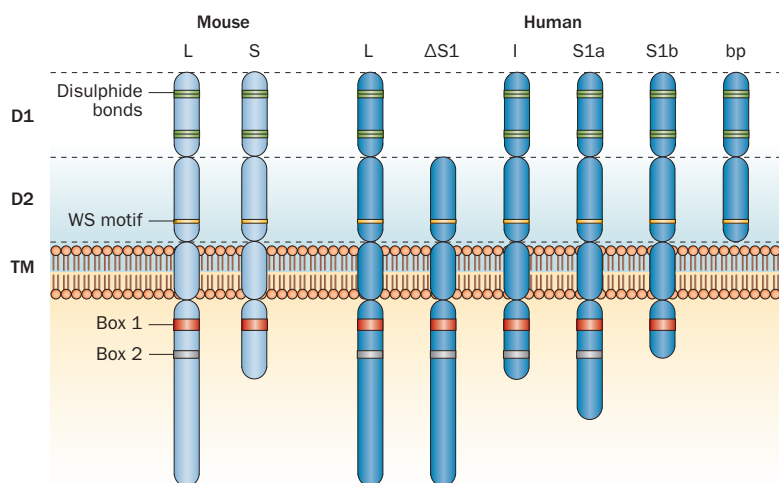


Figure 2 | Mouse and human PRL-R proteins. The PRL-R protein consists of an extracellular domain comprising two binding domains (D1 and D2) and a transmembrane domain that are identical across species, as well as a cytoplasmic domain of variable length and composition. Features such as a disulphide bond and a tryptophan–serine motif, as well as Box 1 and Box 2, are conserved. Multiple isoforms of membrane-bound PRL-R that result from alternative splicing of the primary mRNA transcript have been identified in rodents and humans. Long, short and intermediate isoforms have been characterized. The human $\Delta S1$ isoform represents an mRNA splice variant that lacks the entire D1 domain of the receptor. Both short human PRL-R isoforms (S1a and S1b) seem to be inactive from a signalling perspective, but might serve as ligand traps that function to either internalize ligand and/or downregulate prolactin-induced signalling. A soluble human PRL-R binding protein contains only the extracellular domain. Abbreviations: bp, PRL-R binding protein; D1, binding-domain 1; D2, binding-domain 2; I, intermediate isoform; L, long isoform; PRL-R, prolactin receptor; S, short isoform; TM, transmembrane domain; WS, tryptophan–serine motif.

either alternative splicing of *PRLR* mRNA or cleavage of the membrane receptor.⁴³ The main isoform of PRL-R found in humans is a long near-ubiquitous 598 amino acid protein. Human PRL-R can bind at least three ligands (prolactin, placental lactogen and growth hormone), which makes it difficult to determine the specific effects of prolactin *in vivo*.^{44,45}

Mechanisms of activation

Similar to growth hormone receptor dimers,⁴⁶ human PRL-R dimers are constitutively expressed on the cell surface.^{47,48} These dimers associate via the transmembrane domains. PRL-R homodimers cannot drive signal transmission in the absence of the prolactin ligand and heterodimers of long and short isoforms of human PRL-R are functionally inactive complexes.⁴⁷ PRL-R does not have intrinsic tyrosine kinase activity, but transmits a signal through associated cytoplasmic proteins such as Janus protein kinase 2 (JAK-2).⁴⁹ Signalling is initiated by the binding of a single ligand molecule (prolactin) to two extracellular interaction sites of different affinities called binding-domain 1 (D1) and binding-domain 2 (D2) on predimerized PRL-Rs and triggers a change in the conformation of the receptor dimer.⁵⁰ The molecular mechanism of activation of growth hormone receptor has been described and involves a fascinating multistep scissor-like mechanical model in which conformational changes within the receptor dimer ultimately lead to receptor

activation.⁵¹ A similar mechanism might apply to all class I cytokine receptors, including PRL-R; however, this generalized mechanism remains to be demonstrated.⁵²

Signalling pathways

Although the signalling pathways downstream of the long PRL-R isoform have been well characterized,⁵³ little is known about prolactin actions mediated by the short PRL-R isoform. Prolactin signalling through the long isoform activates many kinases. First, JAK-2 phosphorylates tyrosine residues on the intracellular part of the receptor and autophosphorylates residues within itself. Receptor-associated JAK-2 also phosphorylates cytoplasmic members of the signal transducer and activator of transcription (Stat) family that represent the canonical JAK-2–Stat pathway.⁴ Second, activation of the Src family of tyrosine kinases is required for cell proliferation induced by PRL.^{54,55} For example, proto-oncogene tyrosine-protein kinase Src (commonly known as Src kinase)^{56,57} signalling stimulates expression of the long PRL-R isoform at both the mRNA and protein levels, as well as downstream signalling pathways in the mammary gland.⁵⁸ Furthermore, Src kinase associates with the PRL-R in lipid rafts, where it promotes receptor internalization via dynamin.⁵⁹ Third, phosphatidylinositol 3-kinase (PI3K)/AKT,⁶⁰ mitogen-activated protein kinase (MAPK)³ and serine/threonine kinase Nek3–Vav2–Rac1 pathways⁶¹ are also activated through PRL-R (Figure 3). These signalling events induce several prolactin-responsive genes, such as those encoding proteins involved in cell proliferation (such as cyclin D1 and cytokine-inducible SH2-containing protein) and cell differentiation.^{62,63}

An emergent member of the prolactin signalling cascade, Arf-GAP with GTPase, ANK-repeat and PH-domain-containing protein 2 (commonly known as PI3-kinase enhancer-A; PIKE-A), associates directly with both STAT5 and PRL-R, which is an essential event for prolactin-induced activation of STAT5 and subsequent gene transcription.⁶⁴ Interactions between PRL-R and the kinases or other proteins involved in positive or negative signal regulation (such as adapters, phosphatases and suppressors of cytokine signalling), have been mapped to regions of the cytoplasmic domain. For example, the tyrosine residues in the C-terminal portion of the long PRL-R isoform contribute to STAT5 engagement and phosphorylation by JAK-2.⁶⁵ The intracellular domain of the human long PRL-R isoform contains 10 tyrosine residues, and phosphorylation of intracellular tyrosines creates binding sites for various members of the Src-homology 2 (SH2) protein family.^{66,67} The various isoforms of PRL-R possess different signalling properties. For example, short PRL-R is not tyrosine-phosphorylated, which prevents this isoform from interacting directly with SH2-containing proteins such as Stat factors.^{36,68,69}

Lessons from animal models

The study of prolactin-deficient and PRL-R-deficient mice,^{70,71} as well as prolactin-overexpressing transgenic mice,⁷² has provided important information on the main prolactin signalling pathway. Although developed

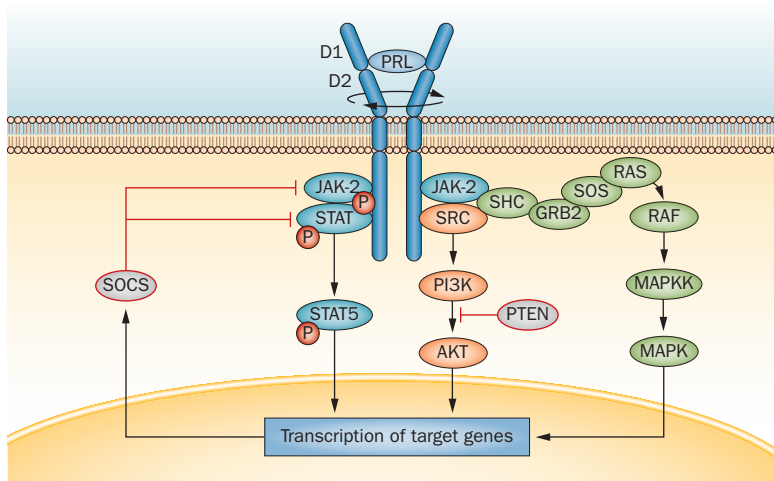


Figure 3 | Major signalling cascades triggered by the long PRL-R isoform. PRL-R exists predominantly as a homodimer held together by the transmembrane helices. Binding of the prolactin hormone ligand converts the receptor into a left-hand crossover state that induces separation of the helices at the lower transmembrane boundary (arrow). Ligand-induced activation of PRL-R triggers several signalling cascades. The main pathway involves the tyrosine kinase JAK-2, which in turn activates STAT5. The MAPK pathway is another important cascade activated by PRL-R and involves the SHC/GRB2/SOS/RAS/RAF intermediaries upstream of MAPK kinases. Recruitment of PI3K leads to AKT activation, and the phosphatase PTEN negatively regulates this pathway. Abbreviations: D1, binding-domain 1; D2, binding-domain 2; Grb2, growth factor receptor-bound protein 2; JAK-2, Janus kinase 2; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; P, phosphate; PI3K, phosphatidylinositol 3-kinase; PRL-R, prolactin receptor; RAF, rat fibrosarcoma virus; RAS, rat sarcoma; SHC, SHC-transforming protein 1; SOCS, suppressor of cytokine signalling; SOS, son of sevenless; Src, proto-oncogene tyrosine-protein kinase Src; Stat, signal transducer and activator of transcription.

independently, different *Stat5* knockout mouse models⁷³ showed a remarkable degree of phenotypic concordance and enabled prolactin functions to be identified *in vivo*. However, the extremely broad spectrum of prolactin activities must be regarded as a panel of functions that are modulated by prolactin rather than being strictly dependent on the hormone.

With the exception of its role in lactation, the human physiological functions of prolactin are poorly understood, largely because of evolutionary adaptations that have led to differences in the biological activity and regulation of this hormone between humans and rodents. However, rodent models that express human prolactin have been developed with the aim of clarifying patterns of prolactin tissue expression.^{74,75} Attributed functions of prolactin include involvement in reproduction and lactation, growth, metabolism, electrolyte transport and behaviour;³⁻⁵ however, it should be noted that some of these functions are species dependent.

One newly described function for prolactin is in proliferation of pancreatic β cells,⁷⁶⁻⁷⁸ in mice, PRL-R is required for β -cell proliferation and maintenance of normal glucose homeostasis during pregnancy.⁷⁹ Heterozygous *Prlr*^{+/-} mice are glucose intolerant during pregnancy and their wild-type offspring are at increased risk of developing glucose intolerance during their own pregnancies.⁸⁰ Thus, normal prolactin activity during pregnancy is important for normal glucose homeostasis in both the present

pregnancy and in pregnancies of future generations. In pregnant wild-type mice, prolactin repressed islet levels of menin and stimulated β -cell proliferation.⁷⁶ These results expand our understanding of the mechanisms that underlie diabetes mellitus pathogenesis and reveal potential targets for therapy in this disease.

A role for prolactin in cartilage physiology has also emerged. A preclinical study performed in rats revealed the protective effects of prolactin against inflammation-induced chondrocyte apoptosis and the therapeutic potential of increasing prolactin levels to reduce permanent joint damage and inflammation in rheumatoid arthritis.⁸¹

Novel roles in human pathophysiology

Anovulatory infertility

Hyperprolactinaemia is a well-established cause of hypogonadotropic hypogonadism and anovulatory infertility,⁸² but the mechanism by which prolactin inhibits hypothalamic secretion of gonadotropin-releasing hormone I (GnRH-I; gonadoliberin-1) was unclear. New evidence has demonstrated that this inhibition involves metastasis-suppressor kisspeptin-1 (also known as KiSS-1) neurons, that express PRL-R.^{83,84} Mice rendered hyperprolactinaemic do not ovulate, have low circulating levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and exhibit reduced hypothalamic expression of the *Kiss1* gene, which encodes kisspeptin-1.⁸⁴ Moreover, kisspeptin-1 immunoreactivity is reduced in both the arcuate nucleus and the anteroventral periventricular nucleus in the hyperprolactinaemic mouse model. Intraperitoneal injections of kisspeptin-1 restored both hypothalamic GnRH-I and gonadotropin secretion, as well as ovarian cyclicity, which suggests that kisspeptin-1 neurons have a major role in hyperprolactinaemic anovulation (Figure 4).⁸⁴ These experiments suggest prolactin-mediated inhibition of GnRH-I occurs, in part, through decreased secretion of kisspeptin-1. Prolactin might also have direct effects on other GnRH-I afferent neurons, and the possibility that other non-neural factors could act on kisspeptin-1 and GnRH-I neuronal secretions cannot be excluded. Nevertheless, this study suggests that kisspeptin-1 administration might be a viable therapeutic approach to restore fertility. Indeed, our own clinical data demonstrate that the gonadal axis can be reactivated by kisspeptin-1 administration in hyperprolactinaemic women who are resistant to or intolerant of dopamine agonists (J. Young unpublished data). Likewise, during lactation, which is a state of physiological hyperprolactinaemia, a selective loss of kisspeptin-1 input to GnRH-I neurons has been observed by others,⁸⁵ and prolactin contributes to the inhibition of kisspeptin-1 in the arcuate nucleus and anteroventral periventricular nucleus.^{86,87}

Familial idiopathic hyperprolactinaemia

Until the past few years, contrary to other anterior pituitary hormones, no human health disorder had been attributed to a mutation in the genes that encode prolactin or its receptor. In late 2013, Newey and colleagues reported the first finding of an inactivating mutation in *PRLR* in three sisters with familial idiopathic

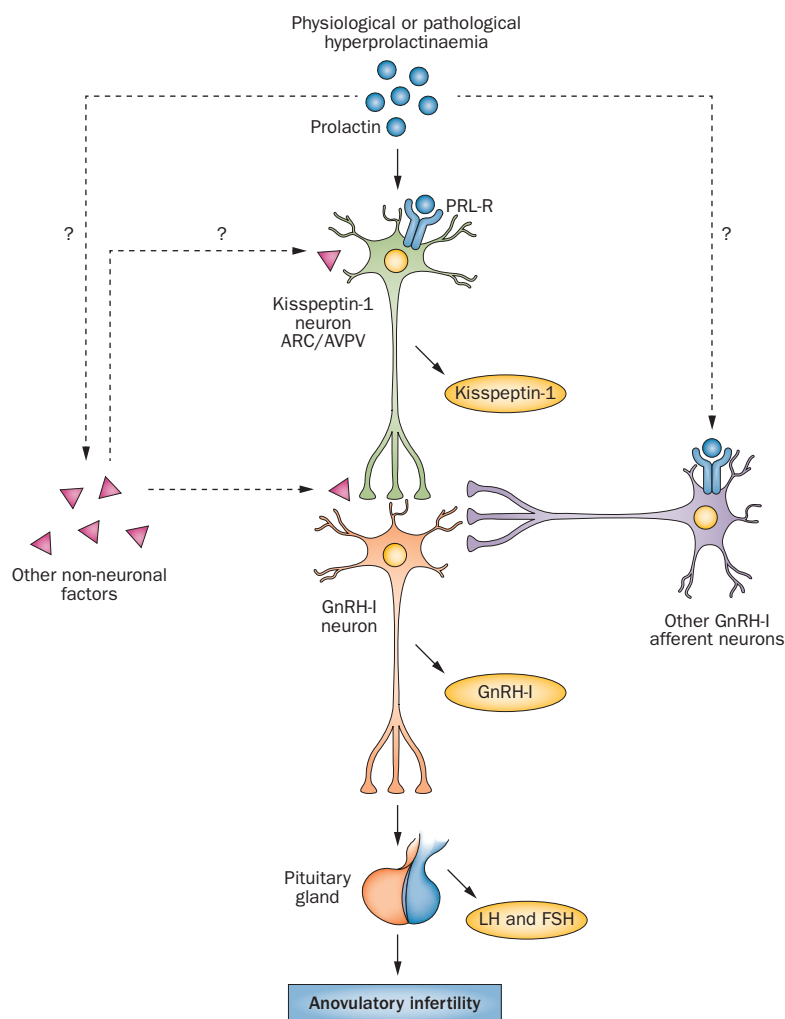


Figure 4 | Model of mechanisms of hyperprolactinaemia-induced anovulatory infertility. As PRL-Rs are not expressed on GnRH-I neurons, hyperprolactinaemia induces infertility via its actions on nearby cells. Increased serum levels of prolactin lead to decreased kisspeptin-1 expression in kisspeptin-1 neurons in both the hypothalamic ARC and AVPV nuclei, which is mediated by PRL-R expressed on these cells. Suppression of kisspeptin-1 reduces secretion of GnRH-I from hypothalamic neurons. This decreased GnRH-I secretion results in reduced LH and FSH secretion and loss of ovarian stimulation, which can result in infertility. Prolactin might also have direct effects on other GnRH-I afferent neurons. Moreover, involvement of other non-neuronal factors affecting kisspeptin-1 and GnRH-I secretion from neurons cannot be excluded. Abbreviations: ARC, arcuate; AVPV, anteroventral periventricular; GnRH-I, gonadotropin-releasing hormone I; LH, luteinizing hormone; PRL-R, prolactin receptor.

hyperprolactinaemia, two of whom had oligomenorrhea and one of whom had infertility.⁸⁸ The index patient had given birth to four children and had been treated with a dopamine agonist at the end of each breastfeed as a result of having persistent galactorrhea. Sequencing analysis of the *PRLR* gene in these patients identified a heterozygous point mutation (c.635A>G) that results in PRL-R His188Arg protein. This mutation seems to inactivate the high-affinity binding site located in the extracellular portion of PRL-R. *In vitro* studies have shown that the PRL-R His188Arg mutation reduces phosphorylation of factors involved in the canonical JAK–Stat pathway that is activated following prolactin exposure.⁸⁸

Several hypotheses about effects of this mutation on levels of prolactin and on reproduction have been proposed (Figure 5). This controversial work has generated much discussion in the literature.^{89–91} Although the causal relationship between the loss-of-function mutation in *PRLR* and hyperprolactinaemia phenotype is strong when their cosegregation was taken into consideration, the late onset of hyperprolactinaemia in patients with a germline mutation in *PRLR* was surprising. The authors argue the existence of a delayed phenotype as observed in *PRLR*^{-/-} mice, which is a possibility that cannot be excluded.^{23,88} However, the lack of increased levels of prolactin during childhood in patients carrying the mutation could also be because pituitary lactotroph cells were not exposed to estrogens before puberty. This late onset of manifestation in the patients with a *PRLR* mutation contrasts with the early-onset phenotype that is seen in patients with heterozygous loss-of-function mutations of the growth hormone receptor.⁹²

Several issues exist regarding the effects on reproduction of hyperprolactinaemia that are associated with mutations in the *PRLR* gene. The clinical heterogeneity with regard to fertility in this family is an issue. The variability of the effects of hyperprolactinaemia on the gonadal axis, ovulation and fertility is a well-established fact in women with high levels of prolactin due to different causes,^{82,93} and this family is not an exception. However, an important limitation of this study is the lack of formal demonstration of a causal relationship between high levels of prolactin in the serum and infertility. No data demonstrate that normalization of hyperprolactinaemia leads to restoration of normal menses and fertility. Moreover, the mechanism of this infertility was not discussed in depth. A possible deleterious effect on the endometrium was proposed by the authors;⁸⁸ however, the data in the literature indicate that the direct impact of hyperprolactinaemia on ovarian and endometrial functions is marginal in humans.⁹⁴ In addition, a lack of galactorrhea is a particular feature of two of the patients with mutated *PRLR*, which was probably a result of the existence of resistance to prolactin in the milk ducts; whereas the persistent galactorrhea in the proband is puzzling. Finally, it is somewhat questionable that a simple heterozygous loss-of-function *PRLR* mutation could lead to hyperprolactinaemia and clinical manifestations. However, loss-of-function mutations in the gene encoding the growth hormone receptor are known to be capable of causing growth hormone insensitivity syndrome, and heterozygous loss-of-function mutations have been shown to have a dominant negative effect in this setting.^{92,95} The authors of this article emphasized the possibility that, because of the heterozygous mutational status, the proportion of wild-type or mutated PRL-R homodimers or heterodimers might vary from one tissue or patient to another.⁸⁸ Contrary to these observations, mice with heterozygous mutations in *Prlr* do not exhibit infertility or galactorrhea, or have abnormal levels of circulating prolactin,⁹⁶ but they are unable to breastfeed their offspring.^{3,97} Despite these outstanding questions, this study is the first to describe the existence of

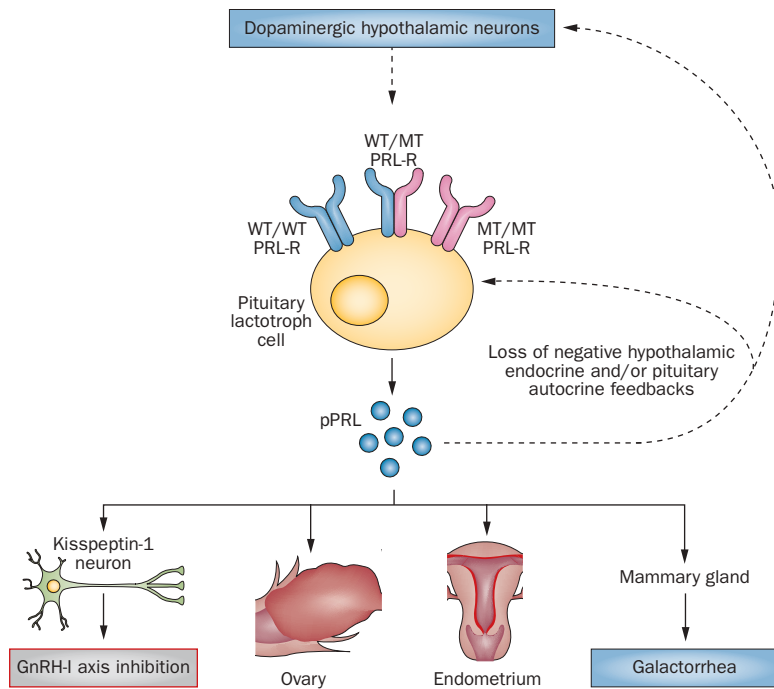


Figure 5 | Possible pathological effects of the PRL-R His188Arg heterozygous loss-of-function mutation on circulating levels of prolactin and on reproductive phenotypes.⁸⁸ Patients carrying the PRL-R His188Arg mutation present with hyperprolactinaemia as a result of loss of negative feedback of pPRL on either hypothalamic tuberoinfundibular dopaminergic neurons and/or lactotroph cells. Given that the mutation is heterozygous, the ratio of wild-type or mutated PRL-R homodimers or heterodimers might differ between various tissues or from patient to another. This variability could, in part, explain the clinical heterogeneity observed in this family with regard to both fertility and galactorrhea. The infertility in two women with the mutation could be a consequence of anovulation induced by inhibition of kisspeptin-1 neurons. Although unlikely in women, direct ovarian and/or endometrial deleterious effects of excess prolactin cannot be formally excluded. Abbreviations: GnRH-I, gonadotropin-releasing hormone I; MT, mutant; pPRL, pituitary prolactin; PRL-R, prolactin receptor; WT, wild-type.

an inactivating germline mutation in *PRLR*, which adds a genetic aetiology to the already long list of causes of hyperprolactinaemia (Box 1).

Elsewhere, a non-synonymous gain-of-function *PRLR* variant (that results in a PRL-R Ile146Leu mutation) that affects the extracellular domain of the protein and results in increased basal JAK-2–Stat5 signalling *in vitro* has been reported in 5.6% of women with benign breast fibroadenomas.⁹⁸ However, this Ile146Leu variant has also been reported as a common polymorphism that occurs in ~2.4% of European American populations,⁹⁹ which argues against any role of this variant in this disorder.

Tumorigenesis

Apart from hyperprolactinaemia, which is the most widely characterized disorder in humans that is related to prolactin signalling, the role of prolactin and PRL-R in the initiation and/or progression of tumours remains an active area of debate. Some studies have attempted to address the role of prolactin in promoting breast cancer using cellular and molecular studies or transgenic rodent models.^{100–104} Epidemiological studies have shown conflicting results.^{105–108} Indeed, large prospective cohort

studies reported a significantly increased occurrence of breast cancer when comparing top to bottom quartiles of normal serum prolactin levels in postmenopausal women;^{105,106} but a non-significant increased risk in premenopausal women.^{106,107} Moreover, other groups consider that this risk is confined to postmenopausal women taking hormone replacement therapy.¹⁰⁸ Furthermore, the clinical relevance of these epidemiological studies is limited by observations that serum levels of prolactin in the study participants remained within the normal range and were not associated with clinical symptoms. To our knowledge, no interventional study has demonstrated that lowering levels of prolactin reduces the occurrence of breast cancer. Concerning women with obvious hyperprolactinaemia, two studies failed to show an association of this state with the risk of breast cancer.^{109,110} One study showed no increase in the number of breast cancers detected prior to diagnosis of hyperprolactinaemia.¹⁰⁹ Another study involving patients with hyperprolactinaemia treated with a dopamine agonist showed no excess risk of breast cancer.¹¹⁰ Moreover, studies investigating associations between genetic variants of *PRL* and *PRLR* and breast cancer are limited, but favour the lack of an association.¹¹¹ Thus, the relevance of pPRL to the pathophysiology of human breast tumours remains nothing less than controversial.

In addition to endocrine pPRL secreted by anterior pituitary lactotroph cells, ePRL of local origin could also contribute to tumorigenesis in an autocrine fashion.⁵⁶ Indeed, autocrine ePRL could provide locally derived mitogenic and prosurvival stimuli, as well as induce cell migration and facilitate angiogenesis;^{56,112} however, this hypothesis remains controversial. Of note, a study of 160 human breast adenocarcinomas showed considerable expression of the PRL-R protein in only four tumours, which indicates that PRL-R is rarely overexpressed in human breast malignancies.¹¹³ Additionally, a study of *PRL* gene expression in 144 human breast tumours showed very low or undetectable mRNA transcript levels in the majority of samples,¹¹⁴ which suggests that autocrine and/or paracrine ePRL signalling is not a general mechanism that promotes breast cancer cell growth. These findings that breast tumours do not express ePRL differ considerably from those reported previously;^{102,103} however, these discrepancies might be attributable to differing sensitivities of the experimental techniques used in the studies.

Autocrine and/or paracrine ePRL has also been suggested to contribute to prostate tumorigenesis. *PRL* and *PRLR* are expressed in normal and tumour human prostate tissues;¹¹⁵ mice that overexpress *PRL* in the prostate develop hyperplasia, intraepithelial neoplasia and even prostatic adenocarcinoma.^{72,116} Epidemiologically, the presence of prolactin and phosphorylated STAT5 in human prostate tumours correlates with high tumour grade and aggressive disease course.^{117,118} Autocrine and/or paracrine prolactin-mediated activation of STAT5 might provide an attractive new pathway for targeting in the treatment of prostate cancer. The first evidence for efficacy of pharmacological targeting of STAT5a/b to inhibit

castrate-resistant growth of prostate cancer has been reported,¹¹⁹ which supports a case for clinical development of STAT5a/b inhibitors as therapies for advanced prostate cancers.

Despite the uncertainty, strategies for suppressing the actions of prolactin in breast and prostate cancer therapies are emerging. The underlying theory is that pure PRL-R antagonists could prevent the pro-tumoural actions of endogenous prolactin by competing for receptor binding sites;¹²⁰ however, no trials of PRL-R antagonists in patients have been reported. As alternatives to prolactin antagonists, antibodies directed against the extracellular juxta-membrane domain could prevent active conformation of the receptor or disrupt the PRL-R–JAK2 interaction. For example, a high potency humanized neutralizing monoclonal antibody that is directed against the extracellular domain of PRL-R has been developed.¹²¹ This antibody inhibited PRL-R function *in vivo* in a preclinical prolactin-sensitive tumour model.¹²¹ A phase I trial to evaluate the clinical utility of this antibody in patients with breast or prostate cancer is underway.¹²²

16 kDa PRL

Angiogenesis

Unlike the main 23 kDa active isoform of prolactin, which is proangiogenic, the 16 kDa prolactin inhibits angiogenesis,¹²³ and, thus, could have antitumoural and antimetastatic actions.^{124–126} The mechanisms by which 16 kDa prolactin exerts its antiangiogenic activities are manifold.¹²⁷ First, 16 kDa prolactin inhibits activation of the MAPK pathway¹²⁸ and induces cell cycle arrest of endothelial cells.¹²⁹ Additionally, 16 kDa prolactin triggers apoptosis of endothelial cells,¹³⁰ prevents endothelial cell migration by inhibiting the Ras–Tiam1–Rac1–Pak1 signalling pathway¹³¹ and reduces activation of nitric oxide synthase endothelial, thus preventing vasodilation.¹³² Finally, 16 kDa prolactin promotes endothelial inflammation by enhancing leukocyte adhesion and also inhibits maturation of blood vessels.^{133,134} Surprisingly, 16 kDa prolactin does not signal via PRL-R, and its endothelial binding site was unknown until last year. Plasminogen activator inhibitor-1 (PAI-1), which inhibits the fibrinolytic agents tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), is a binding partner of 16 kDa prolactin.¹³⁵ By signalling through the PAI-1/uPA/uPA receptor cell surface complex, 16 kDa prolactin impairs tumour vascularization and growth, but also promotes thrombolysis by inhibiting the anti-fibrinolytic activity of PAI-1.¹³⁵ In view of the pro-angiogenic and prothrombotic properties of the tumour milieu, the antiangiogenic and profibrinolytic activities of 16 kDa prolactin might be an attractive target for antitumour therapy.

In addition to its potential antitumoural effects, 16 kDa prolactin is involved in the control of ocular angiogenesis¹³⁶ and seems to inhibit the progression of diabetic retinopathy in rodents.^{137,138} Decreased serum levels of 16 kDa prolactin in patients with diabetes mellitus could contribute to the development and progression of diabetic retinopathy.¹³⁹

Box 1 | Main causes of hyperprolactinaemia

Physiological

Pregnancy
Lactation
Nipple stimulation

Analytical

Macroprolactinemia

Pathological

Prolactinomas and mixed secreting adenomas
Hypothalamic and pituitary stalk disorders

- Compressive macroadenoma
- Hypophysitis
- Granulomatous disease
- Rathke cleft cyst
- Irradiation and/or trauma
- Tumours (craniopharyngiomas, germinoma, metastases)

Medication

- Dopamine antagonists (antipsychotics, anti-emetics, α -methyl dopa antihypertensive)
- Other (antidepressants, estrogens, opiates)

Chronic renal failure
Ectopic prolactin secretion¹⁵¹

- Ovarian dermoids
- Hypernephroma
- Bronchogenic carcinoma

Genetic

- *PRLR* loss-of-function mutation

Idiopathic

Unknown

Peripartum cardiomyopathy

The 16 kDa isoform of prolactin could also be a major factor in the initiation and progression of peripartum cardiomyopathy (PPCM),^{19,140,141} as well as in the onset of preeclampsia.¹⁴² PPCM is a potentially life-threatening cardiac disorder that can occur in previously healthy women towards the end of pregnancy or in the first postpartum months.¹⁴⁰ Hilkkiker-Kleiner and colleagues studied a mouse model of PPCM, in which Stat3 was specifically deleted from cardiomyocytes (CKO mice), cardiac expression of cathepsin D was increased and led to increased generation of the 16 kDa isoform.¹⁹ This cleavage reaction is favoured by high levels of 23 kDa prolactin during the peripartum period and by the physiological increase in oxidative stress during gestation.¹⁴³ Treatment of these mice with bromocriptine (a dopaminergic agonist that causes a decrease in prolactin secretion) during gestation prevented the onset of PPCM, whereas overexpression of cathepsin D resulted in augmentation of deterioration of the cardiac capillary network and its function.¹⁹ These data strongly support a causative role of 16 kDa prolactin in this disorder, at least in mice, and have opened up new perspectives in clinical research, as patients with PPCM have elevated serum levels of cathepsin D and 16 kDa prolactin.¹⁹

A small pilot study of patients who had recovered from a first episode of PPCM provided evidence that bromocriptine could prevent PPCM recurrence during subsequent pregnancies.^{19,144} The same treatment might also be beneficial in the acute phase of PPCM.^{145–148} Thus, bromocriptine could be a novel disease-specific treatment for

PPCM, but randomized trials with large numbers of participants are needed before this approach can be recommended as a routine strategy.¹⁴⁹ In the same CKO mouse model, most of the deleterious effects of 16 kDa prolactin on endothelial cells were attributed to the activity of microRNA-146a (miR-146a).¹⁵⁰ Although 16 kDa prolactin has few direct effects on cardiomyocytes, this isoform induces endothelial cells to release miR-146a-loaded exosomes, which can enter cardiomyocytes. Exosome-derived miR-146a reduces cardiomyocyte metabolic activity and impairs endothelial-to-cardiomyocyte communication via the neuregulin and epidermal growth factor receptor signalling pathways.¹⁵⁰ Furthermore, plasma levels of miR-146a are elevated in patients with PPCM,¹⁵⁰ which suggests that this miRNA transcript could be used as a biomarker and/or a new therapeutic target for this disorder.

Conclusions

Major advances in our understanding of the mechanisms of action of prolactin and its receptor, as well as their roles in human disease have emerged in the past

decade. For example, we now have a better knowledge of the hypothalamic effects of hyperprolactinaemia. The newly described numerous extrapituitary sites of prolactin secretion, as well as the identification of functions for the 16 kDa isoform, extend the functional scope of this hormone. In addition, the first loss-of-function germline mutation in the gene encoding PRL-R in the context of familial idiopathic hyperprolactinaemia has expanded our knowledge. Finally, the development of adjuvant therapies to target prolactin and/or PRL-R with a focus in oncology and cardiology opens up new perspectives for this 'old' hormone.

Review criteria

A search for original articles published between 1984 and 2014, focusing on prolactin receptor, was performed in PubMed. The search terms used were "prolactin receptor", "prolactin action", "16 kDa prolactin" "growth hormone receptor" and specific combinations of prolactin and clinical syndromes deriving from these searches. The resulting references, including reviews, were used as leads for further literature searches.

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Author contributions

V.B. wrote the manuscript and researched data for the article. N.B., J.Y. and P.C. provided substantial contribution to discussions of the content. All authors contributed to reviewing and/or editing the manuscript before submission.