The Complex Genetics of Kallmann Syndrome: *KAL1*, *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, et al.

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Key Words

Anosmia · Cell migration defect · Genetic disease · Hypogonadotropic hypogonadism · Kallmann syndrome · Neuroendocrine GnRH cells · Olfactory bulb development · Renal aplasia

Abstract

Kallmann syndrome (KS) combines hypogonadotropic hypogonadism and anosmia. Anosmia is related to the absence or hypoplasia of the olfactory bulbs and tracts. Hypogonadism is due to gonadotropin-releasing hormone (GnRH) deficiency, which presumably results from a failure of the embryonic migration of neuroendocrine GnRH cells from the olfactory epithelium to the forebrain. This failure could be a consequence of the early degeneration of olfactory nerve and terminal nerve fibres, because the latter normally act as guiding cues for the migration of GnRH cells. Defects in GnRH cell fate specification, differentiation, axon elongation or axon targeting to the hypothalamus median eminence may, however, also contribute to GnRH deficiency, at least in some genetic forms of the disease. To date, five KS genes have been identified, namely, *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, and *KAL1*. Mutations in these genes, however, account for barely 30% of all KS cases. Mutations in *FGFR1*, encoding fibroblast growth factor receptor 1, underlie an autosomal dominant form of the disease. Mutations in *PROKR2* and *PROK2*, encoding prokineticin receptor-2 and prokineticin-2,

have been found in heterozygous, homozygous or compound heterozygous states. These two genes are likely to be involved both in monogenic recessive and digenic or oligogenic KS transmission modes. Finally, *KAL1*, encoding the extracellular glycoprotein anosmin-1, is responsible for the X chromosome-linked form of the disease. It is believed that anosmin-1 acts as an enhancer of FGF signalling and perhaps of prokineticin signalling too.

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Clinical Description of Kallmann Syndrome

Maestre de San Juan, a Spanish anatomist, was probably the first to report the association of the absence of olfactory structures in the brain and the presence of small testes in an individual [Maestre de San Juan, 1856]. The syndrome was identified as a clinical entity in 1944 by an American medical geneticist, Kallmann, who carried out a study on the occurrence of hypogonadism accompanied by anosmia in three affected families [Kallmann et al., 1944]. He showed the cosegregation of anosmia and hypogonadism in all the affected individuals, and therefore established that this syndrome can be hereditary. In the 1950s, de Morsier, an anatomist from Switzerland, further documented this syndrome [de Morsier, 1954, 1962; de Morsier and Gauthier, 1963]. He described the underdevelopment or absence of the olfactory bulbs and

tracts in several male patients with hypogonadism. These olfactory anomalies can now be shown by magnetic resonance imaging (MRI) [Klingmüller et al., 1987]. The occasional presence of various cerebral or cranial anomalies was also reported [Gauthier, 1960]. Some years later, the hypogonadism was ascribed to GnRH deficiency [Naftolin et al., 1971], which can be estimated in the patients by using endocrinological tests [see Seminara et al., 1998]. The degree of hypogonadism and of smell deficiency may vary significantly within affected families [Santen and Paulsen, 1972; Lieblisch et al., 1982; and see pedigrees in Dodé et al., 2003, 2006, 2007; Pitteloud et al., 2005, 2006a, b; Trarbach et al., 2006], even between monozygotic twins [Hermanussen and Sippell, 1985; Hipkin et al., 1990]. In addition, a few cases of seeming reversal of hypogonadism after discontinuation of hormonal treatment have been reported [Pitteloud et al., 2005; Raivio et al., 2007; Ribeiro et al., 2007]. Finally, a variety of other disorders are present in only a fraction of KS patients. These disorders include mirror movements of the upper limbs (bimanual synkinesis) [Conrad et al., 1978; Schwankhaus et al., 1989; Quinton et al., 1996, 2001; Mayston et al., 1997; Krams et al., 1999; Vulliemoz et al., 2005; Tole et al., 2006], eye movement abnormalities [Schwankhaus et al., 1989], congenital ptosis [Hardelin et al., 1993b; Reardon, 2007], abnormal visual spatial attention [Kertzman et al., 1990], hearing impairment [Santen and Paulsen, 1972; Lieblisch et al., 1982; Hill et al., 1992; Coatesworth and Woodhead, 2002], renal agenesis [Wegenke et al., 1975; Hardelin et al., 1993a; Kirk et al., 1994], cleft lip or palate [Santen and Paulsen, 1972; Lieblisch et al., 1982; Molsted et al., 1997; Murray and Schutte, 2004], agenesis of one or several teeth (hypodontia) [de Zegher et al., 1995; Molsted et al., 1997], obesity [Lieblisch et al., 1982], and other less documented anomalies [see Tsai and Gill, 2006 for review].

Diagnosis and Treatment

Most cases are diagnosed in adolescence because of the lack of sexual development, identified by prepubertal testes and absent virilisation in males or the lack of breast development and primary amenorrhea in females. KS is diagnosed when low serum gonadotropins and gonadal steroids are coupled with a compromised sense of smell. The latter should be ascertained by the means of detailed questioning and olfactory screening tests [Rosen et al., 1979; Doty et al., 1984; Davidson and Murphy, 1997; Chalhoui et al., 2005] because it is rarely mentioned sponta-

neously. MRI of the forebrain can be carried out to show hypoplasia or aplasia of the olfactory bulbs and tracts. Notably, KS may also be suspected as early as in infancy in boys, in the presence of cryptorchidism or a micropenis, combined with subnormal LH and FSH concentrations. Indeed, the postnatal surge in FSH, LH, and testosterone in the male infant as a consequence of the continued function of the foetal GnRH pulse generator provides a 6-month window of opportunity to establish the diagnosis of hypogonadotropic hypogonadism [Grumbach, 2005], and alert the clinician to the possibility of its association with olfactory abnormality. In this connection, the usefulness of forebrain MRI in diagnosing the disease in patients too young to undergo meaningful testing of olfaction or of the hypothalamo-pituitary-gonadal axis should be emphasised [Truwit et al., 1993; Birnbacher et al., 1994].

The treatment of KS is that of the hypogonadism. It aims firstly to initiate virilisation or breast development, and secondly to develop fertility. Hormone replacement therapy, with testosterone for males and combined oestrogen and progesterone for females, is the treatment to stimulate the development of secondary sexual characteristics. For those desiring fertility, either gonadotropins or pulsatile GnRH can be used to obtain testicular growth and sperm production in males or ovulation in females. Both treatments restore fertility in a vast majority of affected individuals [Buchter et al., 1998]. It is still unknown whether transient hormone replacement therapy in affected male infants to simulate the postnatal surge in gonadotropins could have later impact on their sexual life and reproductive prognosis [see Grumbach, 2005].

The Complex Genetics of Kallmann Syndrome

The prevalence of the disease has been estimated at 1 out of 8000 in boys. In girls, the prevalence might be five times lower. Although most KS patients present as sporadic cases, many cases are clearly familial, with three modes of inheritance being reported: X chromosome-linked recessive (OMIM #308700), autosomal dominant (OMIM #147950), and autosomal recessive (OMIM #244200) (<http://www.ncbi.nlm.nih.gov/omim/>). In the autosomal dominant form, incomplete penetrance has been emphasised [Santen and Paulsen, 1972; White et al., 1983].

Five causal genes have been identified to date, namely, by chronological order of discovery, *KALI* [Franco et al., 1991; Legouis et al., 1991; Hardelin et al., 1992], *FGFR1*

[Dodé et al., 2003], *PROKR2* and *PROK2* [Dodé et al., 2006] and *FGF8* [Falardeau et al., 2008]. Various loss-of-function mutations in *KAL1*, encoding the extracellular matrix glycoprotein anosmin-1, and in *FGFR1*, encoding fibroblast growth factor receptor-1, underlie the X chromosome-linked form (KAL1) and an autosomal dominant form (KAL2) of KS, respectively. Notably, some of the mutations found in *FGFR1* were de novo mutations [Sato et al., 2006; Zenaty et al., 2006; Dodé et al., 2007]. More recently, putative loss-of-function mutations in *PROK2* and *PROKR2*, encoding prokineticin-2 and prokineticin receptor-2, respectively, have been detected in some KS patients. Most of these mutations are missense mutations, and many are also present in apparently unaffected individuals, thus raising questions regarding their pathogenic role in the disease. For most of the mutations, however, deleterious effects on prokineticin signalling have subsequently been shown in vitro [Cole et al., 2008; C. Monnier, C. Dodé, P. Rondard, unpublished results]. The finding of both heterozygous and homozygous (or compound heterozygous) unrelated patients for given *PROKR2* and *PROK2* mutations [Dodé et al., 2006; Leroy et al., 2008] is quite remarkable and raises the question of a possible digenic or oligogenic mode of inheritance in heterozygous patients. To date, digenic inheritance has been shown in three such patients, who were carrying missense mutations both in *PROKR2* and either *PROK2* [Cole et al., 2008] or *KAL1* [Dodé et al., 2006, and unpublished results]. It is tempting to speculate that the latter two patients have hypomorphic *KAL1* alleles encoding protein variants that would still retain some biological activity, whereas the vast majority of the *KAL1* mutations reported so far are nonsense mutations, frameshift mutations, or large gene deletions, which are expected to inactivate protein synthesis, and are apparently sufficient to produce the abnormal phenotype in males. Other patients carrying heterozygous mutations in *PROKR2*, *PROK2*, or hypomorphic mutations in *KAL1* are expected to carry additional mutations in other, as yet unknown, KS genes. Indeed, mutations in the five known genes together account for barely 30% of KS cases, indicating that other genes responsible for the disease remain to be discovered, some of which might also be involved in FGF signalling or prokineticin signalling. Notably, oligogenic mode of inheritance may also apply to patients carrying mutations in *FGFR1* or *FGF8*. Two patients carrying a monoallelic or a biallelic mutation in *FGF8* have indeed been found to have also mutations in *FGFR1* [Falardeau et al., 2008].

Phenotype-Genotype Correlations

For each genetic form of KS identified so far, the clinical heterogeneity of the disease within affected families clearly indicates that the manifestation of KS phenotypes is dependent on factors other than the mutated gene itself. These factors probably include epigenetic factors and modifier genes, both of which have not yet been identified. In addition, it is possible that oligogenic inheritance accounts in part for the long recognised incomplete penetrance of the disease, at least in some cases. That said, some general features have emerged from clinical observations in the patients affected by the different genetic forms of KS. For instance, a greater variability in the degree of hypogonadism has been observed in patients carrying mutations in *FGFR1*, *FGF8*, *PROKR2* or *PROK2*, than in *KAL1* patients [Oliveira et al., 2001; Dodé et al., 2003, 2006; Falardeau et al., 2008; Salenave et al., 2008]. In particular, spontaneously fertile individuals carrying mutations in any of the four autosomal KS genes account for the transmission of the disease over several generations, while the X-linked form of KS is usually transmitted by the female carriers of *KAL1* mutations, who are clinically unaffected. Among the various non-reproductive and non-olfactory disorders that are observed in a fraction of KS patients [see Tsai and Gill, 2006], some have been reported for specific genetic forms of the disease. For instance, unilateral renal agenesis occurs in approximately 30% of *KAL1* patients [Hardelin et al., 1993a; Kirk et al., 1994], but has not been reported in patients with *FGFR1*, *FGF8*, *PROKR2* or *PROK2* mutations so far. On the other hand, the loss of nasal cartilage, hypoplasia or aplasia of the external ear and anomalies of the fingers or toes have only been reported in *KAL2* patients [Dodé et al., 2003, 2007; Zenaty et al., 2006]. By contrast, tooth agenesis and hearing impairment are common to several genetic forms of KS [Hardelin et al., 1993b; Söderlund et al., 2002; Dodé et al., 2003, 2007; Massin et al., 2003; Sato et al., 2004; Falardeau et al., 2008], although it should be noted that the mechanism (conductive, perceptive, or mixed) of the hearing impairment could vary between different genetic forms. Palate defects should also be considered as one of these shared traits, even though the severity differs between *KAL1* (high arched palate) and other genetic forms, including *KAL2* (cleft palate may occur in as many as 25–30% of the *KAL2* cases [Dodé et al., 2003, 2007; Albuisson et al., 2005; Pitteloud et al., 2006b; Trarbach et al., 2006; Falardeau et al., 2008]). Lastly, bi-manual synkinesis (involuntary upper limb mirror movements) is highly prevalent in *KAL1* (perhaps >75% of the

cases) [Quinton et al., 1996, 2001], but seems to be much less common in *KAL2* [Dodé et al., 2003, 2007; Trarbach et al., 2006]. Additional anomalies have not been reported so far in KS patients carrying mutations in *PROKR2* or *PROK2*, with the notable exception of a severe sleep disorder and marked obesity in one patient [Dodé et al., 2006], which could be related to the known function of prokineticin-2 signalling in behavioural circadian rhythms, including sleep-wake and ingestive behaviour [Cheng et al., 2002, 2006; Negri et al., 2004; Li et al., 2006; Hu et al., 2007; Prosser et al., 2007; for review see Zhou and Cheng, 2005].

Bioactivities of the Kallmann Syndrome Proteins, and Their Functional Interactions

FGFR1 is a member of the receptor tyrosine kinase superfamily. FGF signalling controls cell proliferation, migration, differentiation, and survival, and thus plays essential roles in various processes of embryonic development [Thisse and Thisse, 2005]. In the presence of heparan sulphate proteoglycans (HSPG) [Harmer, 2006], FGF binds with high affinity to FGFR and induces receptor dimerisation, which triggers transautophosphorylation of tyrosine residues in the intracellular domain. A variety of signalling proteins are phosphorylated in response to FGF stimulation (Shc, phospholipase C γ , STAT1, Gab1, FRS2 α ...), leading to activation of downstream signalling pathways that include Ras/Mitogen-activated protein kinase (MAPK) and Phosphatidylinositol-3 kinase/Akt pathways [Eswarakumar et al., 2005]. Different FGFRs have different specificities towards FGF ligands. FGF8 can activate the FGFR1c splice form of FGFR1 [Zhang et al., 2006]. *PROKR2* belongs to the G protein-coupled seven transmembrane domain receptor family. *PROKR2* is one of the two identified receptors to prokineticins-1 and -2 [Lin et al., 2002; Masuda et al., 2002; Soga et al., 2002], a pair of bioactive peptides involved in various processes, including circadian rhythms in the brain, gastrointestinal smooth muscle contraction, and angiogenesis [for review see Zhou and Cheng, 2005]. Alternative splicing of the *PROK2* transcript leads to a larger isoform of prokineticin-2 containing a supplementary 21-amino acid fragment rich in basic residues, which might be processed into a smaller peptide by proteolytic cleavage [Chen et al., 2005]. Prokineticins are believed to bind to heparan sulphate glycosaminoglycans [LeCouter et al., 2003]. Therefore, HSPGs are likely to be involved in prokineticin signalling too. Activation of prokineticin

receptors, through their coupling to various G proteins, leads to mobilisation of intracellular calcium, phosphoinositide hydrolysis, and activation of the MAPK and protein kinase B pathways [Lin et al., 2002; Masuda et al., 2002; and see Malbon, 2005 for review]. Finally, there is increasing evidence that cross-talk between receptor tyrosine kinases and G-protein-coupled receptors can occur both at the most proximal level (receptor transactivation) and at a variety of sites further downstream in signalling cascades [see Gavi et al., 2006; Natarajan and Berk, 2006; Delcourt et al., 2007 for reviews]. Such cross-roads provide a molecular explanation for the integration of intracellular signalling. In the context of their involvement in KS, the FGFR1- and *PROKR2*-signalling pathways could interfere at one or several levels.

KAL1 encodes a 680-amino acid secreted glycoprotein of approximately 95 kDa [see Hardelin, 2001 for review]. The protein has been named anosmin-1 in reference to the compromised sense of smell in KS. Anosmin-1 is a modular protein comprising a large cysteine-rich N-terminal domain, a whey acidic protein (WAP)-like domain, four contiguous fibronectin-like type III (FnIII) repeats, and a small C-terminal domain rich in basic residues. The WAP domain is similar to those of a number of serine protease inhibitors [Schalkwijk et al., 1999; Clauss et al., 2002; Bouchard et al., 2006], whereas the FnIII repeats are structurally related to some cell adhesion molecules [see Brümmendorf and Rathjen, 1993; Potts and Campbell, 1996]. The bioactivities of anosmin-1 at a molecular level, however, have remained elusive. When anosmin-1 is produced in transfected mammalian cells, the bulk of the protein is detected at the cell surface [Rugarli et al., 1996; Soussi-Yanicostas et al., 1996]. The interaction of anosmin-1 with the cell membrane is critically dependent on the presence of heparan sulphate glycosaminoglycans, to which the FnIII domains of the protein bind [Soussi-Yanicostas et al., 1996; Hu et al., 2004; Hudson et al., 2006]. The importance of this molecular interaction for the activity of the protein in vivo has been elegantly confirmed in a model of transgenic nematodes that mis-/overexpress the *KAL1* orthologue in certain neurons: the axon branching phenotype was reversed in mutant worms lacking either heparan 6O-sulphotransferase or C5-epimerase, two enzymes involved in the biosynthesis of heparan sulphate polysaccharide chains [Bülow et al., 2002; Bülow and Hobert, 2004]. During the organogenesis period in man, anosmin-1 is found in basement membranes and interstitial matrices of diverse embryonic structures, and its presence is regionally and temporally restricted [Hardelin et al., 1999]. Anosmin-1

could, possibly through its interaction with HSPGs, stabilize growth factor-receptor complexes (see below), control extracellular matrix turnover, or promote conformational changes of growth factors, to name a few [Tumova et al., 2000; Hacker et al., 2005]. The binding of both FGFR1 and anosmin-1 to HSPG glycosaminoglycan moieties, as well as the various traits (bimanual synkinesis, palate abnormalities, tooth agenesis) shared by a fraction of the KAL1 and KAL2 patients in addition to hypogonadism and anosmia, prompted the proposal that these proteins interact within an extracellular signalling complex [Dodé et al., 2003]. Indeed, anosmin-1 has been shown to enhance the mitogenic effect of the FGFR1c isoform on lymphoid cells in the presence of FGF2 [Gonzalez-Martinez et al., 2004]. Therefore, FGFR1 and anosmin-1 are likely to cooperate in FGF-signalling also in vivo. The precise mechanism of their cooperation, however, is still unknown. Since prokineticins are believed to interact with HSPGs, it is tempting to speculate that anosmin-1 is also involved in prokineticin-signalling.

It is noteworthy that the role played by anosmin-1 in FGF-signalling, and possibly prokineticin-signalling, could explain the lower prevalence of the disease in females. Indeed, the X chromosome-linked recessive mode of inheritance is unlikely to account for the higher prevalence in males, because *KAL1* mutations have been found in approximately 10% of all KS males only. We propose the following alternative explanation, which is based on the observation that *KAL1* partially escapes X inactivation in man [Franco et al., 1991; and see Brown and Greally, 2003 for review] (probably an evolutionary remainder of the ancestral pseudoautosomal location of the gene in lower primates [del Castillo et al., 1992]). As a consequence, girls are expected to synthesise a higher dose of anosmin-1 than boys do. We suggest that a physiologically higher concentration of anosmin-1 in female embryonic tissues could partially compensate for a pathological situation of haploinsufficiency for *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, or other, still unidentified, KS genes, resulting in a lower incidence of the disease in females.

Kallmann Syndrome and CHARGE Syndrome

CHARGE syndrome has an estimated birth incidence of 1 in 8,500–12,000. The defining features that make the acronym are coloboma, heart anomalies, choanal atresia, retardation of growth and/or development, genital and ear anomalies. However, no single feature is universally present or sufficient for the diagnosis of CHARGE syn-

drome, and the degree of severity varies. Other frequently occurring features include characteristic face and hand dysmorphism, hypotonia, arhinencephaly, semi-circular canal agenesis or hypoplasia, deafness, urinary tract anomalies, orofacial clefting, dysphagia, and tracheo-oesophageal anomalies. New diagnosis criteria have been proposed in the past few years [Verloes, 2005; Sanlaville and Verloes, 2007]. Moreover, a recent clinical study has led to the important conclusion that most if not all CHARGE patients have both olfactory bulb aplasia and hypogonadotropic hypogonadism, that is, the two KS defining features [Pinto et al., 2005]. Consequently, previously reported KS cases associated with congenital heart disease [Cortez et al., 1993] or choanal atresia [Klein et al., 1987] could in fact represent unrecognised mild CHARGE cases [Ogata et al., 2006]. CHARGE syndrome shares additional traits with the KAL2 form of KS, including cleft lip or palate, present in 20–35% of KAL2 and CHARGE patients, hypoplasia or aplasia of external ear, noted in many CHARGE patients and a few KAL2 cases [Zenaty et al., 2006], and coloboma, which has been reported in at least one KAL2 patient too [Dodé et al., 2003]. Most CHARGE syndrome individuals are heterozygous for loss-of-function mutations in *CHD7*, encoding a chromodomain (chromatin organization modifier domain) helicase DNA-binding protein [Visser et al., 2004; Lalani et al., 2006]. Because of the similarity between CHARGE and KAL2 phenotypes, it is tempting to speculate that *CHD7* haploinsufficiency results in reduced transcription of *FGFR1* or other genes involved in the FGFR1 signalling pathway. Alternatively, insufficient signalling through FGFR1 could decrease *CHD7* activity. Notably, *Chd7* heterozygous mutant mice [Kiernan et al., 2002; Bosman et al., 2005] and mice homozygous for a hypomorphic allele of *Fgfr1* [Trokovic et al., 2003] not only share palatal defects but also middle ear ossicle anomalies, which further argues for a functional interaction between the two genes.

Pathogenesis of Kallmann Syndrome: Current Hypotheses

Several studies, especially one by the Swiss anatomist de Morsier in the 1950s [de Morsier, 1954, 1962; de Morsier and Gauthier, 1963], led to the conclusion that the smell deficiency in KS is related to a defect in olfactory bulb development, and the hypogonadism was attributed to GnRH deficiency as soon as 1971 [Naftolin et al., 1971]. However, new light was shed on the pathogenesis in the

Fig. 1. Embryonic migration of neuroendocrine gonadotropin-releasing hormone (GnRH) cells. **A** From the olfactory epithelium to the rostral forebrain. Parasagittal section of the nasal region in a 42-day-old human embryo. Rostral is on the left. Two clusters of migrating GnRH cells (arrows) can be seen along the broad swath of neural cell adhesion molecule (NCAM)-immunoreactive nerve fibers that extend from the olfactory epithelium (oe) to the developing olfactory bulb (ob). Scale bar = 100 μ m. (Reproduced from Schwanzel-Fukuda et al. [1996] with permission). **B** Intracerebral path. Horizontal section of the primitive cerebral hemispheres (ch) in a 42- to 44-day-old embryo. Rostral is on the left. In each hemisphere, cords of GnRH-immunoreactive migrating cells (arrows) can be seen in the most superficial zone of the medial neural wall. v: Lateral ventricles. Scale bar = 100 μ m. (Reproduced from Hardelin et al. [1999] with permission).

late 1980s, with the discovery of the close topographical link between the peripheral olfactory system and neuroendocrine GnRH cells during embryonic life, as well as the finding that these cells had not migrated normally in a human foetus affected by X-linked KS (see below). The identification of several genes responsible for KS and the engineering of animal models of the disease, especially mouse models, in the last few years, offer insights into the cellular mechanisms leading to hypogonadism and anosmia, which we will discuss. In addition, the various non-reproductive, non-olfactory anomalies seen in a fraction of the KS patients, at least in the KAL1 and KAL2 genetic forms, indicate that the corresponding KS genes play important roles in many other developmental processes. These include kidney and tooth differentiation, ear and palate morphogenesis, and development of interhemispheric or cortico-spinal axonal tracts. In most if not all cases, however, the precise roles played by KS genes in these processes are still unknown. In this review, we will only briefly discuss renal agenesis, observed in $\sim 30\%$ of the KAL1 patients,

because the mechanism leading to this anomaly involves the mesonephric/Wolffian duct, an embryonic structure that gives rise to the ductus deferens in males.

Disrupted Embryonic Migration of the GnRH Cells in a Foetus Affected by X Chromosome-Linked KS

Embryological studies conducted in mammals [Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989; Ronnekleiv and Resko, 1990; Schwanzel-Fukuda et al., 1996], birds [Murakami et al., 2002], amphibians [Muske and Moore, 1990] and fish [Parhar, 2002] have established that neuroendocrine GnRH cells migrate from the medial part of the olfactory epithelium up to the brain along the olfactory nerve pathway (fig. 1A). In mammals, they migrate in close association with fibres of the vomeronasal and terminal nerves [Pearson, 1941; Schwanzel-Fukuda et al., 1992; Livne et al., 1993; Schwanzel-Fukuda, 1999; Wirsig-Wiechmann et al., 2002; Bless et al., 2005]. On reaching the base of the telencephalon, they penetrate into the brain, just caudal to the olfactory bulb anlage, then make their way superficially along the medial wall of the cerebral hemisphere [Norgren and Brackenbury, 1993; Schwanzel-Fukuda, 1999] (fig. 1B), to the hypothalamic region where they finally settle [Paulin et al., 1977]. Although GnRH cells enter the brain with the central processes of the terminal nerves, there is still some uncertainty as to whether these nerve fibres guide the GnRH cells in their intracerebral navigation [Wray et al., 1994; Wray, 2001; Tobet and Schwarting, 2006]. In man, migration of the GnRH cells begins during the 6th embryonic week [Schwanzel-Fukuda et al., 1996]. This is also the period when axon terminals of the olfactory sensory neurons first come into contact with the rostral pole of the forebrain; this initial contact occurs just before the emergence of the olfactory bulbs [Bossy, 1980; Muller and O'Rahilly, 1989; Gong and Shipley, 1995].

In 1989, the histopathological examination of a 19-week-old male foetus carrying a deletion of the entire KAL1 gene revealed both an absence of the olfactory bulbs and an abnormal location of the neuroendocrine GnRH cells, which were not found in the hypothalamic areas where they were expected. In this foetus, GnRH cells had left the olfactory epithelium, but had accumulated in the upper nasal region, along with the entangled axon terminals of the olfactory, vomeronasal, and terminal nerves, that failed to contact the rostral forebrain [Schwanzel-Fukuda et al., 1989] (fig. 2). These observations seemed to point towards the involvement of KAL1 in the final migration stretch travelled by the GnRH cells and axonal fibres. As a corollary, olfactory bulb aplasia

would be secondary to the absence of a stable contact between the olfactory axons and the forebrain. Indeed, this contact has long been known to be necessary for olfactory bulb morphogenesis to occur [Gong and Shipley, 1995]. The embryonic stage and precise site of the primary defect, however, cannot be deduced from this single snapshot. In particular, it is uncertain whether the olfactory and terminal nerve fibres actually failed to penetrate into the forebrain or degenerated after contact. In any case, the absence of contact between the terminal nerve fibres and the forebrain that was noticed in the KAL1 foetus could explain the failure of GnRH cells to reach the hypothalamic region, because terminal nerve axons are believed to act as mechanical guides for the penetration of these cells into the rostral forebrain [Schwanzel-Fukuda et al., 1992; Livne et al., 1993; and see Schwanzel-Fukuda, 1999 for review].

Olfactory Bulb Aplasia and Other Developmental Defects of the Olfactory System in KS

To date, the murine *Kal1* gene has not yet been identified. Consequently, a mouse model of the KAL1 form of the disease is not available. Antibodies against human anosmin-1 have been produced, which allowed us to study the protein distribution in the early developing olfactory system in man [Hardelin et al., 1999]. During the 6th embryonic week, anosmin-1 is abundant in the interstitial matrix of the presumptive olfactory bulbs, whereas the protein could not be detected either in the olfactory epithelium or along the extracerebral route of elongating olfactory axons. Such a distribution of anosmin-1 was compatible with its proposed role in the short intracerebral navigation of olfactory nerve fibres. It was, however, also consistent with possible direct involvement of the protein in the initial stage of olfactory bulb morphogenesis, which occurs at the end of the 6th embryonic week. The latter possibility was actually reinforced by the finding that anosmin-1 cooperates with FGFR1 in FGF signalling [Gonzalez-Martinez et al., 2004]. Indeed, in conditional knock-out mice that lack *Fgfr1* expression in the telencephalon and the olfactory epithelium, the initial contact between olfactory axons and the forebrain does occur, but the olfactory bulbs do not evaginate from the neuroepithelial wall [Hébert et al., 2003]. In normal mice, the primary contact between pioneer olfactory axons and the rostral forebrain, on embryonic day 12, induces local differentiation of the neural precursor cells [Gong and Shipley, 1995]. Olfactory bulb evagination occurs some hours later, probably as a tectonic consequence of the local decrease in cell proliferation in comparison with sur-

Fig. 2. Failed migration of GnRH-cells in a KAL1 foetus. Parasagittal section of the upper nasal region in a 19-week-old male foetus affected by the X chromosome-linked form of Kallmann syndrome (counterstained with cresyl violet). Clusters of GnRH-immunoreactive cells (in brown) can be seen between the dorsal surface of ethmoid bone cribriform plate (CP) and the meninges (M). No GnRH cells were found in the brain of this foetus. Scale bar = 100 μ m. (Reproduced from Schwanzel-Fukuda et al. [1989] with permission).

rounding areas of the telencephalon where cell division continues for some time. In contrast, in *Fgfr1* knock-out mice, a large proportion of the neuroepithelial cells in the rostral forebrain keep on dividing [Hébert et al., 2003]. Thus, one might predict that the absence of olfactory bulbs in KAL2 patients results from primary morphogenesis failure. That said, one should keep in mind that unlike the mouse model, patients with *FGFR1* mutations have reduced FGF signalling in all tissues, not just in the telencephalon and olfactory epithelium. As a consequence, the effects of such *FGFR1* deficiency on olfactory system development could be less specific in the patients, depending on whether other tissues supporting olfactory bulb development are also affected. In particular, there are inductive interactions between the fronto-nasal mesenchyme and the epithelium that shape the formation of the olfactory pathway, and are influenced by FGF signalling [LaMantia et al., 2000]. *Fgf8*-deficient mice due to a homozygous hypomorphic mutation in *Fgf8* also exhibit olfactory bulb aplasia [Meyers et al., 1998]. Although the underlying embryonic defect has not yet been analysed in detail, recent results suggest that the targeting of early olfactory axons to the forebrain could be abnormal in these mice [Chung et al., 2008].

Mutant mice lacking a functional prokineticin receptor-2 (*Prokr2*^{-/-} mice) have been produced. In these mice,

the olfactory bulbs did not evaginate properly. In addition, the olfactory axons apparently did not reach the forebrain and formed a large tangled sphere-shaped structure in the upper nasal region [Matsumoto et al., 2006]. Therefore, the olfactory bulb hypoplasia found in *Prokr2*^{-/-} mice could be the consequence of the absence of contact between the olfactory axons and the rostral forebrain, a situation reminiscent of the observations made in the KAL1 human foetus (see above). Unfortunately, the *PROKR2* expression profile in the developing olfactory system has not yet been determined in man, which prevents further pathophysiological comparison at the present time. In mutant mice defective for prokineticin-2 (*Prok2*^{-/-} mice), abnormal architecture of the olfactory bulbs with reduced number of granule cells has been reported, but this was attributed to a failure at a late stage of olfactory bulb differentiation, that is, the migration of subventricular zone-derived neuronal progenitors to the granule and periglomerular cell layers [Ng et al., 2005]. However, recent results argue in favour of a role of prokineticin-2 signalling at earlier stages of olfactory bulb differentiation. *Prok2* is indeed expressed in the ependymal and subependymal layers of the early developing olfactory bulbs, at embryonic day 13 in the mouse [Zhang et al., 2007]. It is a target gene of the proneural basic helix-loop-helix transcription factors neurogenin-1 and Mash1 that are involved in olfactory bulb neurogenesis [Zhang et al., 2007]. Finally, *Prok2* was able to enhance the differentiation of adult neural progenitors into neurons in vitro [Zhang et al., 2007]. Moreover, a reanalysis of *Prok2*^{-/-} mice has shown the likely failure of olfactory and terminal nerve axons to reach the forebrain [Piteloud et al., 2007].

The roles played by KS genes in the olfactory system may not be restricted to the development of the olfactory bulbs. Indeed, a study in rodent embryos indicates that anosmin-1 is also involved in the projection of olfactory bulb output neurons to the cerebral cortex. In the rat, axons of the lateral olfactory tract sprout collaterals just before invasion into the cortex. These collaterals represent the only connection between the olfactory bulbs and the cortex, and their formation requires the presence of anosmin-1 [Soussi-Yanicostas et al., 2002]. Notably, poor connectivity between olfactory bulbs and the cortex could account for the compromised olfactory function in some KS individuals who have apparently normal olfactory bulbs, at least by MRI analysis. It is currently unknown, however, whether other KS genes participate in this branching process.

GnRH Cell Migration Defect and Other Developmental Defects of the GnRH Neuroendocrine System in KS

The putative failure in the process of GnRH cell migration, which has been proposed to underlie hypogonadotropic hypogonadism in KS, is not well understood. According to the observations made in the human KAL1 foetus and prokineticin-2 or prokineticin receptor-2 knock-out mice, it may be secondary to the disorganisation of the terminal nerve central roots, which normally guide these cells as they penetrate into the forebrain. Based on in vitro and ex vivo experiments [Cariboni et al., 2004; and see below], however, more direct mechanisms affecting the GnRH neurons themselves have been proposed [for review see Tsai and Gill, 2006]. Finally, hypomorphic and transgenic mouse models have provided some clues to the etiology of the abnormal reproductive phenotype in KAL2. The various animal models of KS are discussed below, with respect to GnRH neuroendocrine system development.

In the absence of a knock-out mouse model of the KAL1 form of the disease to date, gene knockdown strategies have been taken in the zebrafish and medaka teleost fish. As a consequence of partial genome duplication, these species possess two different *kall* genes, *kall1a* and *kall1b* [Ardouin et al., 2000; Okubo et al., 2006]. Knockdown of *kall1a*, but not *kall1b*, resulted in the accumulation of endocrine GnRH cells in the olfactory region and loss of their ability to migrate into the forebrain [Whitlock et al., 2005; Okubo et al., 2006]. These observations demonstrated that *kall1a* is crucial for the migration of these cells, but not for their fate specification within the olfactory epithelium. Although a precise description of concomitant anomalies of the olfactory bulbs and olfactory/terminal nerve fibres in the knockdown fishes was lacking in the original descriptions, it is reasonable to assume that the GnRH cell migration defect could be the result of disrupted development of the olfactory system [Whitlock et al., 2005].

There are presently no pathohistological data to verify that the migration of GnRH cells in KAL2 human embryos is arrested. *Fgfr1*-deficient or *Fgf8*-deficient mice due to homozygous hypomorphic mutations in the corresponding genes have a markedly decreased number or a complete absence of GnRH cells in the hypothalamic region, respectively. Moreover, in the *Fgf8* hypomorphs, GnRH cells never emerge from the olfactory epithelium [Chung et al., 2008; Falardeau et al., 2008]. Notably, additional results in the mouse indicate that the reproductive phenotype in KAL2 may involve defects other than

GnRH cell migration. In particular, ex vivo studies have shown that FGF-signalling is required for GnRH neuronal fate specification, promotes GnRH neurite outgrowth, and provides cues for GnRH neurons to correctly target their axons to the hypothalamus median eminence for hormone release [Gill et al., 2004; Tsai et al., 2005; Gill and Tsai, 2006]. Moreover, targeted expression of an *Fgfr1* dominant negative construct to GnRH-neurons in transgenic mice resulted both in a 30% reduction in the population of hypothalamic GnRH neurons, which was tentatively attributed to reduced cell survival [Tsai et al., 2005], and in a significant reduction in the projections of their axons to the median eminence [Gill and Tsai, 2006]. Although these animals are initially fertile, they display reproductive anomalies, such as delayed puberty, decreased fecundity, and early reproductive senescence. Therefore, migratory arrest of neuroendocrine GnRH cells is unlikely to be the only cause of the GnRH deficiency in KAL2. As mentioned earlier, KAL2 patients exhibit great variability in the degree of hypogonadism, indicating the arrival of at least some GnRH cells in the hypothalamic region of the less severely affected individuals, and presumably a wider spectrum of GnRH system defects in KAL2 than in KAL1.

Prokr2^{-/-} mice, in addition to their olfactory bulb dysgenesis [Ng et al., 2005] and altered circadian rhythms [Li et al., 2006; Hu et al., 2007], also have an abnormal reproductive phenotype [Pitteloud et al., 2007]. Indeed, homozygous mutant mice of both sexes are infertile and have hypoplastic reproductive organs. Moreover, a marked decrease in the number of GnRH cells present in the preoptic area of the brain has been shown, which could be attributed to a defect in the embryonic migration of these cells. In E13 *Prokr2*^{-/-} embryos, GnRH neurons, after crossing the cribriform plate, seemed to be trapped in a spherical-shaped structure, presumably formed by olfactory and terminal nerve axons that did not reach their targets [Pitteloud et al., 2007]. Moreover, the few GnRH neurons that are present in the preoptic area of the *Prokr2*^{-/-} mice may not be functional, consistent with their sexual immaturity. Indeed, *Prokr2* also acts as an output molecule from the suprachiasmatic nucleus circadian clock [Cheng et al., 2002], and therefore could play a role in GnRH pacemaking. A severe atrophy of the reproductive organs has been shown in the *Prokr2*^{-/-} mice of both sexes too, and GnRH neurons were not detected in the hypothalamic region of adult mutants [Matsumoto et al., 2006]. The location of these cells during the embryonic period remains to be determined, but the observation in E12–E13 mutant embryos, that the olfactory and

Fig. 3. Anosmin-1 in embryonic kidneys. Left: Longitudinal section of the mesonephros at embryonic week 7. Anosmin-1 immunoreactivity is detected in the epithelium and basement membrane of the collecting tubules and mesonephric/Wolffian duct (md), but not in the paramesonephric/Müllerian duct (pd). Right: In the metanephros at the same embryonic age, anosmin-1 is present in ureteric bud proximal branches (u) in the medulla. In contrast, the protein is not detected in the cortical zone, either in distal ureteric branches or in differentiating secretory tubules (t). Scale bars = 50 μ m. (Reproduced from Hardelin et al. [1999] with permission).

terminal nerve axons failed to contact the forebrain and were entangled in the upper nasal region suggests that the GnRH cells did not migrate properly in the absence of *Prokr2* as well. In addition, *Prokr2* and *Prokr2* are expressed in the preoptic area and median eminence [Cheng et al., 2006], and therefore could also be involved in the maturation/survival of the GnRH neurons once they have reached their target region.

Renal Aplasia in KS

Differentiation of the permanent kidney (metanephros) is critically dependent on the ureteric bud, an outgrowth of the mesonephric duct, which invades the adjacent metanephric mesenchyme cell cluster at the end of the 5th embryonic week. Reciprocal inductive signalling between the ureteric bud and the metanephric mesenchyme leads to multiple iterations of branching and elongation of the ureteric bud to form the collecting system [for review see Shah et al., 2004], while the mesenchyme is induced to condensate and epithelialise around the branched tips. Based on the detection of anosmin-1 both in the mesonephric duct and ureteric bud branches, but not metanephric tubules, in 6- to 7-week-old human embryos (fig. 3), the unilateral (sometimes bilateral) renal aplasia found in approximately 30% of the KAL1 patients, occasionally combined with ipsilateral absence of the ductus deferens [Kirk et al., 1994], has been attributed to a defect in the formation or branching of the ureteric bud. It is noteworthy that kidney anomalies have not been reported in KAL2 patients, which is consistent with the fact that conditional knock-out mice lacking *Fgfr1* expression

either in the ureteric bud or in the metanephric mesenchyme do not have apparent renal abnormalities [Zhao et al., 2004; Poladia et al., 2006].

Conclusion

Although KS was first identified as a hereditary disease more than 60 years ago, the genetics of this developmental disorder is still incompletely understood, including its much higher prevalence in males than in females. Five KS genes have been identified so far. Despite the fact

that several genes underlying the disease remain to be discovered, KS already appears to result from insufficient cell signalling through FGFR1 or PROKR2. Therefore, KS is a promising 'natural model' to study quantitative aspects of FGF-signalling and prokineticin-signalling in olfactory bulb morphogenesis and neuroendocrine GnRH cell development, as well as the expected interplay between the two signalling pathways. This research may benefit from engineered animal models, especially mouse models including double heterozygous mutants defective for different KS genes.

References

- Albuissou J, Pêcheux C, Carel J, Lacombe D, Leheup B, et al: Kallmann syndrome: 14 novel mutations in *KAL1* and *FGFR1* (*KAL2*). *Hum Mutat* 25:98–99 (2005).
- Ardouin O, Legouis R, Fasano L, David-Watine B, Korn H, et al: Characterization of the two zebrafish orthologues of the *KAL-1* gene underlying X chromosome-linked Kallmann syndrome. *Mech Dev* 90:89–94 (2000).
- Birnbaumer R, Wandl-Vergesslich K, Frisch H: Diagnosis of X-recessive Kallmann syndrome in early infancy. Evidence of hypoplastic rhinencephalon. *Eur J Pediatr* 153: 245–247 (1994).
- Bless EP, Walker HJ, Yu KW, Knoll JG, Moenter SM, et al: Live view of gonadotropin-releasing hormone containing neuron migration. *Endocrinology* 146:463–468 (2005).
- Bosman EA, Penn AC, Ambrose JC, Kettleborough R, Stemple DL, Steel KP: Multiple mutations in mouse *Chd7* provide models for CHARGE syndrome. *Hum Mol Genet* 14: 3463–3476 (2005).
- Bossy J: Development of olfactory and related structures in staged human embryos. *Anat Embryol (Berl)* 161:225–236 (1980).
- Bouchard D, Morisset D, Bourbonnais Y, Tremblay GM: Proteins with whey-acidic-protein motifs and cancer. *Lancet Oncol* 7:167–174 (2006).
- Brown C, Grealis J: A stain upon the silence: genes escaping X inactivation. *Trends Genet* 19:432–438 (2003).
- Brümmendorf T, Rathjen F: Axonal glycoproteins with immunoglobulin- and fibronectin type III related domains in vertebrates: structural features, binding activities, and signal transduction. *J Neurochem* 61:1207–1219 (1993).
- Buchter D, Behre HM, Kliesch S, Nieschlag E: Pulsatile GnRH or human chorionic gonadotropin/human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. *Eur J Endocrinol* 139:298–303 (1998).
- Bülöw H, Hobert O: Differential sulfations and epimerization define heparan sulfate specificity in nervous system development. *Neuron* 41:723–736 (2004).
- Bülöw HE, Berry KL, Topper LH, Peles E, Hobert O: Heparan sulfate proteoglycan-dependent induction of axon branching and axon misrouting by the Kallmann syndrome gene *kal-1*. *Proc Natl Acad Sci USA* 99: 6346–6351 (2002).
- Cariboni A, Pimpinelli F, Colamarino S, Zaninetti R, Piccolella M, et al: The product of X-linked Kallmann's syndrome gene (*KAL1*) affects the migratory activity of gonadotropin-releasing hormone (GnRH)-producing neurons. *Hum Mol Genet* 13:2781–2791 (2004).
- Chalouhi C, Faulcon P, Le Bihan C, Hertz-Pannier L, Bonfils P, Abadie V: Olfactory evaluation in children: application to the CHARGE syndrome. *Pediatrics* 116:e81–e88 (2005).
- Chen J, Kuei C, Sutton S, Wilson S, Yu J, et al: Identification and pharmacological characterization of prokineticin 2 beta as a selective ligand for prokineticin receptor 1. *Mol Pharmacol* 67:2070–2076 (2005).
- Cheng MY, Bullock CM, Li C, Lee AG, Bermak JC, et al: Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* 417:405–410 (2002).
- Cheng MY, Leslie FM, Zhou QY: Expression of prokineticins and their receptors in the adult mouse brain. *J Comp Neurol* 498:796–809 (2006).
- Chung WC, Moyle SS, Tsai PS: Fibroblast growth factor 8 signaling through Fgf receptor 1 is required for the emergence of gonadotropin-releasing hormone neurons. *Endocrinology* 149:4997–5003 (2008).
- Clauss A, Lilja H, Lundwall A: A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to whey acidic protein. *Biochem J* 368:233–242 (2002).
- Coatesworth AP, Woodhead CJ: Conductive hearing loss associated with Kallmann's syndrome. *J Laryngol Otol* 116:125–126 (2002).
- Cole LW, Sidis Y, Zhang C, Quinton R, Plummer L, et al: Mutations in prokineticin 2 (*PROK2*) and *PROK2* receptor (*PROKR2*) in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. *J Clin Endocrinol Metab* 93:3551–3559 (2008).
- Conrad B, Kriebel J, Hetzel WD: Hereditary bi-manual synkinesis combined with hypogonadotropic hypogonadism and anosmia in four brothers. *J Neurol* 218:263–274 (1978).
- Cortez AB, Galindo A, Arensman FW, Van Dop C: Congenital heart disease associated with sporadic Kallmann syndrome. *Am J Med Genet* 46:551–554 (1993).
- Davidson TM, Murphy C: Rapid clinical evaluation of anosmia. The alcohol sniff test. *Arch Otolaryngol Head Neck Surg* 123:591–594 (1997).
- de Morsier G: Etudes sur les dysraphies crâniocéphaliques. 1. Agénésie des lobes olfactifs (telencephaloschizis latéral) et des commissures calleuse et antérieure (telencephaloschizis médian). La dysplasie olfacto-génitale. *Schweiz Arch Neurol Psychiat* 74:309–361 (1954).
- de Morsier G: Median cranioencephalic dysraphias and olfactogenital dysplasia. *World Neurol* 3:485 (1962).
- de Morsier G, Gauthier G: La dysplasie olfacto-génitale. *Pathol Biol* 11:1267–1272 (1963).
- de Zegher F, Lagae L, Declercq D, Vinckier F: Kallmann syndrome and delayed puberty associated with agenesis of lateral maxillary incisors. *J Craniofac Genet Dev Biol* 15:87–89 (1995).
- del Castillo I, Cohen-Salmon M, Blanchard S, Lutfalla G, Petit C: Structure of the X-linked Kallmann syndrome gene and its homologous pseudogene on the Y chromosome. *Nat Genet* 2:305–310 (1992).

- Delcourt N, Bockaert J, Marin P: GPCR-jacking: from a new route in RTK signalling to a new concept in GPCR activation. *Trends Pharmacol Sci* 28:602–607 (2007).
- Dodé C, Levilliers J, Dupont J-M, De Pape A, Le Dû N, et al: *FGFR1* loss-of-function mutations cause autosomal dominant Kallmann syndrome. *Nat Genet* 33:463–465 (2003).
- Dodé C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, et al: Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* 2:1648–1652 (2006).
- Dodé C, Fouveaut C, Mortier G, Janssens S, Bertherat J, et al: Novel *FGFR1* sequence variants in Kallmann syndrome, and genetic evidence that the *FGFR1c* isoform is required in olfactory bulb and palate morphogenesis. *Hum Mutat* 28:97–98 (2007).
- Doty R, Shaman P, Dann M: Development of the University of Pennsylvania Smell Identification Test, a standardized microencapsulated test of olfactory function. *Physiol Behav* 32:489–502 (1984).
- Eswarakumar VP, Lax I, Schlessinger J: Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev* 16:139–149 (2005).
- Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, et al: Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. *J Clin Invest* 118:2822–2831 (2008).
- Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, et al: A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature* 353:529–536 (1991).
- Gauthier G: La dysplasie olfacto-génitale (Agénésie des lobes olfactifs avec absence de développement gonadique à la puberté. *Acta Neurol (Wien)* 21:345–394 (1960).
- Gavi S, Shumay E, Wang HY, Malbon CC: G-protein-coupled receptors and tyrosine kinases: crossroads in cell signaling and regulation. *Trends Endocrinol Metab* 17:48–54 (2006).
- Gill JC, Tsai PS: Expression of a dominant negative FGF receptor in developing GNRH1 neurons disrupts axon outgrowth and targeting to the median eminence. *Biol Reprod* 74:463–472 (2006).
- Gill J, Moenter S, Tsai P: Developmental regulation of gonadotropin-releasing hormone neurons by FGF signaling. *Endocrinology* 145:3830–3839 (2004).
- Gong Q, Shipley MT: Evidence that pioneer olfactory axons regulate telencephalon cell cycle kinetics to induce the formation of the olfactory bulb. *Neuron* 14:91–101 (1995).
- Gonzalez-Martinez D, Kim S, Hu Y, Guimond S, Schofield J, et al: Anosmin-1 modulates fibroblast growth factor receptor 1 signaling in human gonadotropin-releasing hormone olfactory neuroblasts through a heparan sulfate-dependent mechanism. *J Neurosci* 24:10384–10392 (2004).
- Grumbach MM: A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab* 90:3122–3127 (2005).
- Hacker U, Nybakken K, Perrimon N: Heparan sulphate proteoglycans: the sweet side of development. *Nat Rev Mol Cell Biol* 6:530–541 (2005).
- Hardelin J-P: Kallmann syndrome: towards molecular pathogenesis. *Mol Cell Endocrinol* 179:75–81 (2001).
- Hardelin J-P, Levilliers J, del Castillo I, Cohen-Salmon M, Legouis R, et al: X chromosome-linked Kallmann syndrome: stop mutations validate the candidate gene. *Proc Natl Acad Sci USA* 89:8190–8194 (1992).
- Hardelin J-P, Levilliers J, Blanchard S, Carel J-C, Leutenegger M, et al: Heterogeneity in the mutations responsible for X chromosome-linked Kallmann syndrome. *Hum Mol Genet* 2:373–377 (1993a).
- Hardelin J-P, Levilliers J, Young J, Pholsena M, Legouis R, et al: Xp22.3 deletions in isolated familial Kallmann's syndrome. *J Clin Endocrinol Metab* 76:827–831 (1993b).
- Hardelin J-P, Juliard AK, Moniot B, Soussi-Yanicostas N, Verney C, et al: Anosmin-1 is a regionally restricted component of basement membranes and interstitial matrices during organogenesis: implications for the developmental anomalies of X chromosome-linked Kallmann syndrome. *Dev Dyn* 215:26–44 (1999).
- Harmer NJ: Insights into the role of heparan sulphate in fibroblast growth factor signalling. *Biochem Soc Trans* 34:442–445 (2006).
- Hébert JM, Partanen J, Rossant J, McConnell SK: FGF signaling through *FGFR1* is required for olfactory bulb morphogenesis. *Development* 130:1101–1111 (2003).
- Hermanussen M, Sippell WG: Heterogeneity of Kallmann's syndrome. *Clin Genet* 28:106–111 (1985).
- Hill J, Elliott C, Colquhoun I: Audiological, vestibular and radiological abnormalities in Kallmann's syndrome. *J Laryngol Otol* 106:530–534 (1992).
- Hipkin L, Casson I, Davis J: Identical twins discordant for Kallmann's syndrome. *J Med Genet* 27:198–199 (1990).
- Hu WP, Li JD, Zhang C, Boehmer L, Siegel JM, Zhou QY: Altered circadian and homeostatic sleep regulation in prokineticin 2-deficient mice. *Sleep* 30:247–256 (2007).
- Hu Y, Gonzalez-Martinez D, Kim S, Bouloux P: Cross-talk of anosmin-1, the protein implicated in X-linked Kallmann's syndrome, with heparan sulphate and urokinase-type plasminogen activator. *Biochem J* 384:495–505 (2004).
- Hudson ML, Kinnunen T, Cinar HN, Chisholm AD: *C. elegans* Kallmann syndrome protein KAL-1 interacts with syndecan and glypican to regulate neuronal cell migrations. *Dev Biol* 294:352–365 (2006).
- Kallmann FJ, Schoenfeld WA, Barrera SE: The genetic aspects of primary eunuchoidism. *Am J Mental Deficiency* XLVIII:203–236 (1944).
- Kertzman C, Robinson DL, Sherins RJ, Schwankhaus JD, Mc Clurkin JW: Abnormalities in visual spatial attention in men with mirror movements associated with isolated hypogonadotropic hypogonadism. *Neurology* 40:1057–1063 (1990).
- Kiernan AE, Erven A, Voegelings S, Peters J, Nolan P, et al: ENU mutagenesis reveals a highly mutable locus on mouse chromosome 4 that affects ear morphogenesis. *Mamm Genome* 13:142–148 (2002).
- Kirk J, Grant D, Besser G, Shalet S, Smith C, et al: Unilateral renal aplasia in X-linked Kallmann's syndrome. *Clin Genet* 46:260–262 (1994).
- Klein VR, Friedman JM, Brookshire GS, Brown OE, Edman CD: Kallmann syndrome associated with choanal atresia. *Clin Genet* 31:224–227 (1987).
- Klingmüller D, Duwes W, Krahe T, Brecht G, Schweikert H-U: Magnetic resonance imaging of the brain in patients with anosmia and hypothalamic hypogonadism (Kallmann's syndrome). *J Clin Endocrinol Metab* 65:581–584 (1987).
- Krams M, Quinton R, Ashburner J, Friston K, Frackowiak R, et al: Kallmann's syndrome: mirror movements associated with bilateral corticospinal tract hypertrophy. *Neurology* 52:816–822 (1999).
- Lalani SR, Safiullah AM, Fernbach SD, Harutyunyan KG, Thaller C, et al: Spectrum of *CHD7* mutations in 110 individuals with CHARGE syndrome and genotype-phenotype correlation. *Am J Hum Genet* 78:303–314 (2006).
- LaMantia AS, Bhasin N, Rhodes K, Heemskerk J: Mesenchymal/epithelial induction mediates olfactory pathway formation. *Neuron* 28:411–425 (2000).
- LeCouter J, Lin R, Tejada M, Frantz G, Peale F, et al: The endocrine-gland-derived VEGF homologue Bv8 promotes angiogenesis in the testis: Localization of Bv8 receptors to endothelial cells. *Proc Natl Acad Sci USA* 100:2685–2690 (2003).
- Legouis R, Hardelin J-P, Levilliers J, Claverie JM, Compain S, et al: The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell* 67:423–435 (1991).
- Leroy C, Fouveaut C, Leclercq S, Jacquemont S, Boullay HD, et al: Biallelic mutations in the prokineticin-2 gene in two sporadic cases of Kallmann syndrome. *Eur J Hum Genet* 16:865–868 (2008).
- Li JD, Hu WP, Boehmer L, Cheng MY, Lee AG, et al: Attenuated circadian rhythms in mice lacking the prokineticin 2 gene. *J Neurosci* 26:11615–11623 (2006).
- Lieblich JM, Rogol AD, White BJ, Rosen SW: Syndrome of anosmia with hypogonadotropic hypogonadism (Kallmann syndrome). *Am J Med* 73:506–519 (1982).

- Lin DC, Bullock CM, Ehlert FJ, Chen JL, Tian H, Zhou QY: Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor. *J Biol Chem* 277:19276–19280 (2002).
- Livne I, Gibson MJ, Silverman AJ: Biochemical differentiation and intercellular interactions of migratory Gonadotropin-Releasing Hormone (GnRH) cells in the mouse. *Dev Biol* 159:643–656 (1993).
- Maestre de San Juan A: Falta total de los nervios olfatorios con anosmia en un individuo en quien existia una atrofia congenita de los testiculos y miembro viril. *Siglo Medico* 131:211 (1856).
- Malbon CC: G proteins in development. *Nat Rev Mol Cell Biol* 6:689–701 (2005).
- Massin N, Pêcheux C, Eloit C, Bensimon J-L, Galey J, et al: X chromosome-linked Kallmann syndrome: clinical heterogeneity in three siblings carrying an intragenic deletion of the *KAL-1* gene. *J Clin Endocrinol Metab* 88:2003–2008 (2003).
- Masuda Y, Takatsu Y, Terao Y, Kumano S, Ishibashi Y, et al: Isolation and identification of EG-VEGF/prokineticins as cognate ligands for two orphan G-protein-coupled receptors. *Biochem Biophys Res Commun* 293:396–402 (2002).
- Matsumoto S, Yamazaki C, Masumoto KH, Naganano M, Naito M, et al: Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. *Proc Natl Acad Sci USA* 103:4140–4145 (2006).
- Mayston MJ, Harrison LM, Quinton R, Stephens JA, Krams M, Bouloux PM: Mirror movements in X-linked Kallmann's syndrome. I. A neurophysiological study. *Brain* 120:1199–1216 (1997).
- Meyers EN, Lewandoski M, Martin GR: An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. *Nat Genet* 18:136–141 (1998).
- Molsted K, Kjaer I, Giwercman A, Versterhauge S, Skakkebaek N: Craniofacial morphology in patients with Kallmann's syndrome with and without cleft lip and palate. *Cleft Palate-Craniofacial J* 34:417–424 (1997).
- Muller F, O'Rahilly R: The human brain at stage 17, including the appearance of the future olfactory bulb and the first amygdaloid nuclei. *Anat Embryol* 180:353–369 (1989).
- Murakami S, Seki T, Arai Y: Structural and chemical guidance cues for the migration of GnRH neurons in the chick embryo. *Prog Brain Res* 141:31–44 (2002).
- Murray J, Schutte B: Cleft palate: players, pathways, and pursuits. *J Clin Invest* 113:1676–1678 (2004).
- Muske L, Moore FL: Ontogeny of gonadotropin-releasing hormone neuronal systems in amphibians. *Brain Res* 534:177–187 (1990).
- Naftolin F, Harris GW, Bobrow M: Effect of purified luteinizing hormone releasing factor on normal and hypogonadotropic anovulatory men. *Nature* 232:496–497 (1971).
- Natarajan K, Berk BC: Crosstalk coregulation mechanisms of G protein-coupled receptors and receptor tyrosine kinases. *Methods Mol Biol* 332:51–77 (2006).
- Negri L, Lattanzi R, Giannini E, De Felice M, Colucci A, Melchiorri P: Bv8, the amphibian homologue of the mammalian prokineticins, modulates ingestive behaviour in rats. *Br J Pharmacol* 142:181–191 (2004).
- Ng KL, Li JD, Cheng MY, Leslie FM, Lee AG, Zhou QY: Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. *Science* 308:1923–1927 (2005).
- Norgren RB, Brackenbury R: Cell adhesion molecules and the migration of LHRH neurons during development. *Dev Biol* 160:377–387 (1993).
- Ogata T, Fujiwara I, Ogawa E, Sato N, Udaka T, Kosaki K: Kallmann syndrome phenotype in a female patient with CHARGE syndrome and *CHD7* mutation. *Endocr J* 53:741–743 (2006).
- Okubo K, Sakai F, Lau EL, Yoshizaki G, Takeuchi Y, et al: Forebrain gonadotropin-releasing hormone neuronal development: insights from transgenic medaka and the relevance to X-linked Kallmann syndrome. *Endocrinology* 147:1076–1084 (2006).
- Oliveira LMB, Seminara SB, Beranova M, Hayes FJ, Valkenburgh SB, et al: The importance of autosomal genes in Kallmann syndrome: Genotype-phenotype correlations and neuroendocrine characteristics. *J Clin Endocrinol Metab* 86:1532–1538 (2001).
- Parhar IS: Cell migration and evolutionary significance of GnRH subtypes. *Prog Brain Res* 141:3–17 (2002).
- Paulin C, Dubois M, Barry J, Dubois P: Immunofluorescence study of LH-RH producing cells in the human fetal hypothalamus. *Cell Tissue Res* 182:341–345 (1977).
- Pearson AA: The development of the nervus terminalis in man. *J Comp Neurol* 75:39–66 (1941).
- Pinto G, Abadie V, Mesnage R, Blustajn J, Cabrol S, et al: CHARGE syndrome includes hypogonadotropic hypogonadism and abnormal olfactory bulb development. *J Clin Endocrinol Metab* 90:5621–5626 (2005).
- Pitteloud N, Acierno J, Meysing A, Dwyer A, Hayes F, Crowley W: Reversible Kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the *FGFR1* gene. *J Clin Endocrinol Metab* 90:1317–1322 (2005).
- Pitteloud N, Acierno JS Jr, Meysing A, Eliseenkova AV, Ma J, et al: Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA* 103:6281–6286 (2006a).
- Pitteloud N, Meysing A, Quinton R, Acierno JS Jr, Dwyer AA, et al: Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol* 254–255:60–69 (2006b).
- Pitteloud N, Zhang C, Pignatelli D, Li JD, Raivio T, et al: Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA* 104:17447–17452 (2007).
- Poladia DP, Kish K, Kutay B, Hains D, Kegg H, et al: Role of fibroblast growth factor receptors 1 and 2 in the metanephric mesenchyme. *Dev Biol* 291:325–339 (2006).
- Potts J, Campbell I: Structure and function of fibronectin modules. *Matrix Biol* 15:313–320 (1996).
- Prosser HM, Bradley A, Chesham JE, Ebling FJ, Hastings MH, Maywood ES: Prokineticin receptor 2 (Prokr2) is essential for the regulation of circadian behavior by the suprachiasmatic nuclei. *Proc Natl Acad Sci USA* 104:648–653 (2007).
- Quinton R, Duke V, de Zoysa P, Platts A, Valentine A, Kendall B: The neuroradiology of Kallmann's syndrome: a genotypic and phenotypic analysis. *J Clin Endocrinol Metab* 81:3010–3017 (1996).
- Quinton R, Duke VM, Robertson A, Kirk JMW, Matfin G, et al: Idiopathic gonadotrophin deficiency: Genetic questions addressed through phenotypic characterization. *Clin Endocrinol* 55:163–174 (2001).
- Raivio T, Falardeau J, Dwyer A, Quinton R, Hayes FJ, et al: Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med* 357:863–873 (2007).
- Reardon W: Kallmann syndrome presenting as congenital ptosis in brothers. *Clin Dysmorphol* 16:207–208 (2007).
- Ribeiro RS, Vieira TC, Abucham J: Reversible Kallmann syndrome: report of the first case with a *KAL1* mutation and literature review. *Eur J Endocrinol* 15:285–290 (2007).
- Ronnekleiv OK, Resko JA: Ontogeny of gonadotropin-releasing hormone-containing neurons in early fetal development of Rhesus macaques. *Endocrinology* 126:498–511 (1990).
- Rosen S, Gann P, Rogol A: Congenital anosmia: detection thresholds for seven odorant classes in hypogonadal and eugonadal patients. *Ann Otol Rhinol Laryngol* 88:288 (1979).
- Rugarli EI, Ghezzi C, Valsecchi V, Ballabio A: The Kallmann syndrome gene product expressed in COS cells is cleaved on the cell surface to yield a diffusible component. *Hum Mol Genet* 5:1109–1115 (1996).
- Salenave S, Chanson P, Bry H, Pugeat M, Cabrol S, et al: Kallmann's syndrome: a comparison of the reproductive phenotypes in men carrying *KAL1* and *FGFR1/KAL2* mutations. *J Clin Endocrinol Metab* 93:758–763 (2008).

- Sanlaville D, Verloes A: CHARGE syndrome: an update. *Eur J Hum Genet* 15:389–399 (2007).
- Santen RJ, Paulsen CA: Hypogonadotropic eunuchoidism. I. Clinical study of the mode of inheritance. *J Clin Endocrinol Metab* 36:47–54 (1972).
- Sato N, Katsumata N, Kagami M, Hasegawa T, Hori N, et al: Clinical assessment and mutation analysis of Kallmann syndrome 1 (*KALI*) and fibroblast growth factor receptor 1 (*FGFR1*, or *KAL2*) in five families and 18 sporadic patients. *J Clin Endocrinol Metab* 89:1079–1088 (2004).
- Sato N, Ohyama K, Fukami M, Okada M, Ogata T: Kallmann syndrome: somatic and germline mutations of the fibroblast growth factor receptor 1 gene in a mother and the son. *J Clin Endocrinol Metab* 91:1415–1418 (2006).
- Schalkwijk J, Wiedow O, Hirose S: The trappin gene family: proteins defined by an N-terminal transglutaminase substrate domain and a C-terminal four-disulphide core. *Biochem J* 340:569–577 (1999).
- Schwankhaus JD, Currie J, Jaffe MJ, Rose SR, Sherins RJ: Neurologic findings in men with isolated hypogonadotropic hypogonadism. *Neurology* 39:223–226 (1989).
- Schwanzel-Fukuda M: Origin and migration of luteinizing hormone-releasing hormone neurons in mammals. *Microsc Res Tech* 44:2–10 (1999).
- Schwanzel-Fukuda M, Pfaff DW: Origin of luteinizing hormone-releasing hormone neurons. *Nature* 338:161–164 (1989).
- Schwanzel-Fukuda M, Bick D, Pfaff DW: Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. *Mol Brain Res* 6:311–326 (1989).
- Schwanzel-Fukuda M, Abraham S, Crossin KL, Edelman GM, Pfaff DW: Immunocytochemical demonstration of neural cell adhesion molecule (NCAM) along the migration route of luteinizing hormone-releasing hormone (LHRH) neurons in mice. *J Comp Neurol* 321:1–18 (1992).
- Schwanzel-Fukuda M, Crossin KL, Pfaff DW, Bouloux PMG, Hardelin J-P, Petit C: Migration of Luteinizing Hormone-Releasing Hormone (LHRH) neurons in early human embryos. *J Comp Neurol* 366:547–557 (1996).
- Seminara SB, Hayes FJ, Crowley WFJ: Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): Pathophysiological and genetic considerations. *Endocrine Rev* 19:521–539 (1998).
- Shah MM, Sampogna RV, Sakurai H, Bush KT, Nigam SK: Branching morphogenesis and kidney disease. *Development* 131:1449–1462 (2004).
- Söderlund D, Canto P, Mendez J: Identification of three novel mutations in the *KALI* gene in patients with Kallmann syndrome. *J Clin Endocrinol Metab* 87:2589–2592 (2002).
- Soga T, Matsumoto S, Oda T, Saito T, Hiyama H, et al: Molecular cloning and characterization of prokineticin receptors. *Biochim Biophys Acta* 1579:173–179 (2002).
- Soussi-Yanicostas N, Hardelin J-P, Arroyo-Jimenez M, Ardouin O, Legouis R, et al: Initial characterization of anosmin-1, a putative extracellular matrix protein synthesized by definite neuronal cell populations in the central nervous system. *J Cell Sci* 109:1749–1757 (1996).
- Soussi-Yanicostas N, de Castro F, Julliard AK, Perfettini I, Chédotal A, Petit C: Anosmin-1, defective in the X-linked form of Kallmann syndrome, promotes axonal branch formation from olfactory bulb output neurons. *Cell* 109:217–228 (2002).
- Thisse B, Thisse C: Functions and regulations of fibroblast growth factor signaling during embryonic development. *Dev Biol* 287:390–402 (2005).
- Tobet SA, Schwarting GA: Minireview: recent progress in gonadotropin-releasing hormone neuronal migration. *Endocrinology* 147:1159–1165 (2006).
- Tole S, Gutin G, Bhatnagar L, Remedios R, Herbert JM: Development of midline cell types and commissural axon tracts requires *Fgfr1* in the cerebrum. *Dev Biol* 289:141–151 (2006).
- Trarbach EB, Costa EM, Versiani B, de Castro M, Baptista MT, et al: Novel fibroblast growth factor receptor 1 mutations in patients with congenital hypogonadotropic hypogonadism with and without anosmia. *J Clin Endocrinol Metab* 91:4006–4012 (2006).
- Trokovic N, Trokovic R, Mai P, Partanen J: *Fgfr1* regulates patterning of the pharyngeal region. *Genes Dev* 17:141–153 (2003).
- Truwit CL, Barkovich AJ, Grumbach MM, Martini JJ: MR imaging of Kallmann syndrome, a genetic disorder of neuronal migration affecting the olfactory and genital systems. *Am J Neuroradiol* 14:827–838 (1993).
- Tsai PS, Gill J: Mechanisms of disease: insights into X-linked and autosomal-dominant Kallmann syndrome. *Nat Clin Pract Endocrinol Metab* 2:160–171 (2006).
- Tsai P, Moenter S, Postigo H, El Majdoubi M, Pak T, et al: Targeted expression of a dominant-negative fibroblast growth factor (FGF) receptor in gonadotropin-releasing hormone (GnRH) neurons reduces FGF responsiveness and the size of GnRH neuronal population. *Mol Endocrinol* 19:225–236 (2005).
- Tumova S, Woods A, Couchman JR: Heparan sulfate proteoglycans on the cell surface: versatile coordinators of cellular functions. *Int J Biochem Cell Biol* 32:269–288 (2000).
- Verloes A: Updated diagnostic criteria for CHARGE syndrome: a proposal. *Am J Med Genet A* 133:306–308 (2005).
- Visser LE, van Ravenswaaij CM, Admiraal R, Hurst JA, de Vries BB, et al: Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet* 36:955–957 (2004).
- Vulliemoz S, Raineteau O, Jabaudon D: Reaching beyond the midline: why are human brains cross wired? *Lancet Neurol* 4:87–99 (2005).
- Wegenke JD, Uehling DT, Wear JBJ, Gordon ES, Bargman JG, et al: Familial Kallmann syndrome with unilateral renal aplasia. *Clin Genet* 7:368–381 (1975).
- White BJ, Rogol AD, Brown KS, Lieblisch JM, Rosen SW: The syndrome of anosmia with hypogonadotropic hypogonadism: a genetic study of 18 new families and a review. *Am J Med Genet* 15:417–435 (1983).
- Whitlock KE, Smith KM, Kim H, Harden MV: A role for *foxd3* and *sox10* in the differentiation of gonadotropin-releasing hormone (GnRH) cells in the zebrafish *Danio rerio*. *Development* 132:5491–5502 (2005).
- Wirsig-Wiechmann CR, Wiechmann AF, Eisten HL: What defines the nervus terminalis? Neurochemical, developmental, and anatomical criteria. *Prog Brain Res* 141:45–58 (2002).
- Wray S: Development of luteinizing hormone-releasing hormone neurones. *J Neuroendocrinol* 13:3–11 (2001).
- Wray S, Grant P, Gainer H: Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. *Proc Natl Acad Sci USA* 86:8132–8136 (1989).
- Wray S, Key S, Qualls R, Fueshko SM: A subset of peripherin positive olfactory axons delineates the luteinizing hormone releasing hormone neuronal migratory pathway in developing mouse. *Dev Biol* 166:349–354 (1994).
- Zenaty D, Bretones P, Lambe C, Guemas I, David M, et al: Paediatric phenotype of Kallmann syndrome due to mutations of fibroblast growth factor receptor 1 (FGFR1). *Mol Cell Endocrinol* 254–255:78–83 (2006).
- Zhang C, Ng KL, Li JD, He F, Anderson DJ, et al: Prokineticin 2 is a target gene of proneural basic helix-loop-helix factors for olfactory bulb neurogenesis. *J Biol Chem* 282:6917–6921 (2007).
- Zhang X, Ibrahim OA, Olsen SK, Umehori H, Mohammadi M, Ornitz DM: Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. *J Biol Chem* 281:15694–15700 (2006).
- Zhao H, Kegg H, Grady S, Truong H, Robinson M, et al: Role of fibroblast growth factor receptors 1 and 2 in the ureteric bud. *Dev Biol* 276:403–415 (2004).
- Zhou QY, Cheng MY: Prokineticin 2 and circadian clock output. *FEBS J* 272:5703–5709 (2005).