Cell. Mol. Life Sci. 63 (2006) 2512–2526
1420-682X/06/212512-15
DOI 10.1007/s00018-005-5604-3
© Birkhäuser Verlag, Basel, 2006

Cellular and Molecular Life Sciences

Human Genome & Diseases: Review

Kallmann's syndrome, a neuronal migration defect

A. Cariboni, R. Maggi*

Laboratory of Developmental Neuroendocrinology, Department of Endocrinology, Centre of Excellence on Neurodegenerative Disease (CEND), University of Milan, Via G. Balzaretti, 9, 20133 Milan (Italy), Fax +39 02 50318204, e-mail: roberto.maggi@unimi.it

Received 23 December 2005; received after revision 31 May 2006; accepted 6 July 2006
Online First 4 September 2006

Abstract. Infertility and inability to smell are the phenotypical features of Kallmann's syndrome (KS), a genetic disease which affects 1 in 10,000 males and 1 in 50,000 females, the majority of the cases being sporadic. The molecular pathogenesis of KS is complex but mainly referable to the impairment of olfactory axon development and of the migration of gonadotropin-releasing hormone (GnRH) neurons. Only two different genes have been identified so far as responsible for the disease: KAL1 and KAL2, encoding anosmin-1 and fibroblast growth factor receptor 1 (FGFR1), respectively. In this review we focus our attention on insights evoked by recent studies, which propose a new direct role for anosmin-1 in the migration GnRH neurons, and a fascinating hypothesis of interactions between anosmin-1 and FGFR1 systems.

Keywords. Kallmann, anosmin-1, FGFR1, GnRH, neuron, migration.

Introduction

Kallmann syndrome (KS) is an inherited disorder characterized by the association of hypogonadotropic hypogonadism (HH) and anosmia (partial or complete loss of olfaction) [1]. The condition is the most common form of gonadotropin deficiency and affects 1 in 10,000 males and 1 in 50,000 females [2], the majority of cases being sporadic. Olfactory system abnormalities associated with hypogonadism were first observed in 1856 by Maestre de San Juan, a Spanish pathologist who found no olfactory lobes in a man with underdeveloped genitalia. In 1944, the psychiatrist and genetist Franz Josef Kallmann described the syndrome in three families and hypothesized the inherited nature of this condition [1]. In 1954, de Morsier reported several patients with hypogonadism, anosmia and midline anatomic defects, a syndrome he termed 'olfactogenital dysplasia', and he was the first to suggest a hypothalamic origin for the hypogonadism [3]. If the olfactory bulb defect is responsible for the anosmia, the hypogonadism is in fact secondary to a deficiency of the hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) which leads to a lack of release of the pituitary gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH), which are involved in gonadal maturation and functions [4]; hence, the definition hypogonadotropic hypogonadism.

The earliest clinical manifestation of HH is a micropenis/cryptorchidism, but most patients show failed, or more rarely, arrested, puberty due to low plasma levels of gonadotropins. Anosmia, on the other hand, is often not mentioned by the patient and can only be elucidated by specific tests. Loss of the sense of smell in KS is due to abnormal olfactory system development. Aplasia or hypoplasia of the OBs and tracts has been documented both in autopsies [3] and in nuclear magnetic resonance (NMR) neuroimaging studies of KS patients [5–7]. Additional findings in KS patients include a variety of neu-

* Corresponding author.

rological symptoms, such as synkinesia (mirror movements), eye movement abnormalities, cerebellar ataxia, evoked horizontal nystagmus, sensorineural deafness, spatial attentional abnormalities, spastic paraplegia and mental retardation [1, 8–11]. Somatic defects such as pes cavus, unilateral renal agenesis and cleft lip and palate have also been reported. Several of these abnormalities segregate as mendelian traits in association with KS, suggesting that they may be proper components of the disorder. Nevertheless, HH and anosmia represent the most specific diagnostic features of KS.

Anosmia is a lack of the sense of smell, the sensory modality that mediates the communication between our body and the external environment through the recognition of chemical cues. The olfactory system is unique in several respects and has received a great deal of attention from developmental neurobiologists in recent years [12]. In mammals, olfactory chemosensation begins in sensory neurons located in the olfactory epithelium, which is situated in the upper wall of the nasal cavities, and in the epithelium of the vomeronasal organ (VNO), a tubular structure that opens on the ventral aspect of the nasal septum. The ability of the mammalian brain to perceive a broad spectrum of volatile odorant molecules with extraordinary sensitivity and specificity is dependent on the correct organization of the olfactory receptor neurons-olfactory bulb (OB) connection [13]. Olfactory and vomeronasal neuroepithelia arise during embryonic development from a specialized peripheral epithelium (the olfactory placode); their neurons project axons to the main and the accessory OBs, respectively, where they synapse with dendrites of mitral and tufted cells in specialized structures called 'glomeruli' [12]. Olfactory receptor neurons arise and develop many of their differentiated properties without depending on interactions with brain targets, and with the olfactory bulb in particular [14].

In vertebrates, the olfactory bulb develops from the telencephalic vesicle, and the role of the interaction with olfactory receptor axons in its development is still debated. An influence of the olfactory epithelium in triggering telencephalon and olfactory bulb development has been observed in amphibians [15], where the removal of the olfactory anlagen results in an absent or reduced telencephalic vesicle. However, this relationship has not been deeply analyzed in mammals. For example, in the homozygous *pax-6* mutant, an olfactory bulb-like structure starts to develop in the telencephalic vesicle in the total absence of olfactory nerves [16]. On the other hand, the arrival of olfactory axons does appear to have a dramatic influence on cell kinetics and the rate of precursor differentiation in the OB anlagen [17; for a review see ref. 18]. During normal development, a small subset of early pioneer axons grow into the brain from the olfactory placode and penetrate deep into the target region of the telencephalic vesicle [19, 20].

The earliest axons from the olfactory placode have been suggested to contact a telencephalic region that may already be determined to give rise to the OB, although some level of interaction with the pioneer olfactory axons appears to be needed for normal maturation. On the other hand, if the initial determination of the OB does not need olfactory receptor innervation, the development of glomerular organization depends entirely on the ingrowth of olfactory axons [see ref. 21 for a discussion]. Guidance cues, therefore, must be present in the olfactory epithelium and in the OB to mediate axonogenesis to the forebrain, recognition and invasion of the target and, ultimately, the establishment of a refined spatial map [for a review ref. 22]. During the developmental period, the primary axons of the mitral/tufted cells eventually grow into a very narrow region of the ventrolateral part of the telencephalon, adjacent to the pial surface where they form the lateral olfactory tract (LOT) and target areas in the olfactory cortex, anterior olfactory nucleus, piriform cortex, olfactory tubercle, entorhinal cortex and amygdaloid nuclei.

Interesting is the observation that beside giving rise to the primary olfactory sensory neurons, the olfactory epithelium generates a population of migrating neuroblasts [23], and the special 'olfactory ensheathing' glial cells [24, 25] that migrate with olfactory axons. The biological explanation for the link between HH and anosmia became apparent after the demonstration that GnRH neurons also originate in the olfactory placode and that, during embryonic development, they migrate into the forebrain in association with the olfactory/vomeronasal nerves [26, 27]. Therefore, the current hypothesis is that KS is the result of a defect in migration of both olfactory nerves and GnRH neurons. Confirmation of this postulate came from the pathological examination of a 19-week-old human fetus affected by KS [28]. In this fetus, the olfactory, vomeronasal and terminalis nerves were not in contact with the brain, but terminated their axonogenesis within the meninges in an abnormal neural tangle (Fig. 1).

It must be underlined that this finding comes from a single fetus affected by the X-linked form of KS; many clinical as well as experimental *in vivo* and *in vitro* observations (described in the following sections) are indicative of possibly different pathogenetic mechanisms of the disease; however, all of them variably lead to the characteristic diagnostic symptoms of KS, like HH and anosmia.

GnRH neurons failed to enter the brain and were found in the nasal cavities and on the dorsal surface of the cribriform plate in the tangle formed by olfactory nerves. The restoration of pituitary gonadotropin release by GnRH treatments in KS patients confirms that HH results from hypothalamic GnRH deficiency, rather than from gonadotrope cells or gonadal failures [29].

From a therapeutic point of view, even if no treatments have been found to improve the sense of smell, the release

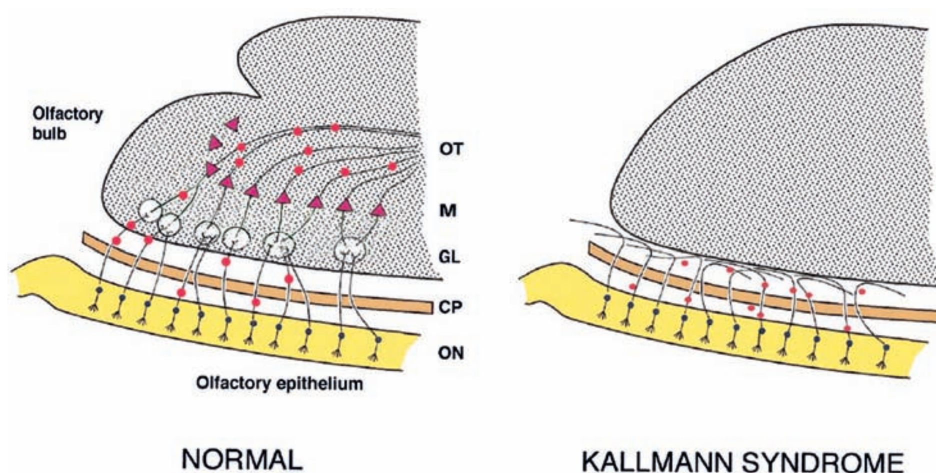

Figure 1. Model for KS pathogenesis. In normal individuals (left), the olfactory neurons (ON) in the olfactory epithelium send their axons through the cribriform plate (CP) of the ethmoid bone to reach the olfactory bulb. Within the glomerular layer (GL) of the bulb, they make synapses with dendrites of mitral cells (M) whose axons will form the olfactory tract (OT). Neurons secreting GnRH (shown in red) originate in the olfactory placode and migrate along the olfactory nerves until they reach the forebrain. In an X-linked KS-affected human fetus (right), the olfactory axons were not targeted to the olfactory bulb, ending their migration between the cribriform plate and the forebrain. GnRH neurons were absent in the brain but they were found clustered on the dorsal surface of the cribriform plate within the dural layers of the meninges [28] [figure adapted from ref. 150; reproduced from the American Journal of Human Genetics, by copyright permission of the University of Chicago Press].

of pituitary gonadotropins can be restored by exogenous subcutaneous or intravenous pulsatile GnRH or gonadotropin treatment [30, 31]. However, by restoring the fertility of patients, treatment may paradoxically lead to a spread of the disease to future generations.

In this review, we analyze KS paying particular attention to the genes involved in the disease and the possible defect in the migration of GnRH neurons.

Genetics of KS

KS is a genetically heterogeneous disease characterized by three modes of familial inheritance, namely X chromosome linked, autosomal dominant and autosomal recessive. However, more than 60% of KS patients present as sporadic cases [11].

The genetic features of the disease became clearer when several reports described kindreds in which hypogonadism and anosmia were part of a complex phenotype, segregating as an X-linked trait [32–34]. In these families, features of KS were associated with other X-linked diseases such as ichthyosis, chondrodysplasia punctata, mental retardation, short stature and ocular albinism. This complex phenotype was found to be due to a deletion on Xp22.3, which involves adjacent disease genes, causing a contiguous gene syndrome [35]. Molecular analysis of deletions and translocations involving this region in male individuals with complex phenotypes made it possible to establish a correlation between the size of the deletion and the clinical features, leading ultimately to the mapping of a KS locus on Xp22.3 [36] and to the ordering of several disease loci in Xp22.3 [37]. Listed in telomere to centromere order, these include short stature, recessive chondrodysplasia punctata, mental retardation, X-linked ichthyosis, KS and ocular albinism. The map assignment of the X-linked KS (X-KS) gene (KAL1; OMIM#308700) to the Xp22.3 region was subsequently confirmed by linkage analysis in families with isolated KS [38]. The KAL1 defects account for approximately 15–20% of total KS [10, 11].

Autosomal dominant as well as recessive transmissions have also been described [11]. Autosomal dominant transmission was proposed in families with incomplete penetrance for the gonadotropin deficiency and isolated anosmia. A gene responsible for an autosomal dominant form of KS has been identified by positional cloning strategies, based on analyses of overlapping chromosomal deletions in patients affected by different contiguous gene syndromes that did or did not include KAL [39]. Dode et al. [39] studied two sporadic cases showing different contiguous gene syndromes that both included KS and they characterized two interstitial deletions within the short arm of chromosome 8. Fine mapping of the deletion defined a new candidate region of 540 kb for autosomal dominant KS that included the putative KAL2 gene (KAL2; OMIM#147950). Among the genes present in this region, the fibroblast growth factor (FGF) receptor 1 (FGFR1) gene was considered the most probable candidate gene, because FGFRs are involved in OB development [40]. Mutation analysis in several families and sporadic cases revealed that this is indeed the case. The frequency of KAL2 mutations is approximately 10% of KS [39, 41].

Depending on the mode of inheritance, the syndrome can occur with a variety of apparently discordant abnormalities, such as upper body mirror movements (X-KS), renal agenesis (X-KS) and midline/craniofacial abnormalities (autosomal KS), including cleft lip and palate. The hypogonadism observed in KAL1 mutations is similar to the gonadotropin deficiency described in KAL2 mutations. However, dental agenesis and cleft palate seem to be specific to the KAL2 defect [41]. Unilateral renal agenesia was only reported for the KAL1 mutation, while bimanual synkinesia appears to be present in both X-linked and autosomal dominant KS [39]. The phenotypic expressivity of congenital isolated HH is very variable. The phenotype may be limited to anosmia in subjects bearing KAL2 or KAL1 mutations. Such variability might depend directly on OB development [42]

Although KS with autosomal recessive inheritance has been described (KAL3; OMIM#244200) [8, 43, 44], no genes responsible for this form have yet been characterized. These forms typically include families with KS in which probands of both sexes were equally affected and parents were phenotypically normal. Cleft lip and palate occurred in both eugonadal and hypogonadal persons with anosmia [8]. Segregation analysis was consistent with autosomal recessive inheritance with variable expression.

The KAL1 gene encodes for anosmin-1

KAL1 is the gene responsible for the KS with an X-linked mode of inheritance. In 1991, two separate groups [45, 46] identified it by positional cloning techniques on a critical region of human chromosome Xp22.3. The gene spans 11 exons and encodes for a secreted protein called anosmin-1 of a predicted molecular mass of 100 kDa.

The orthologues of the human KAL1 gene have been cloned in many species, including *Drosophila* [47] (accession number AF342988), *Caenorhabditis elegans* [48, 49], zebrafish [50], medaka [51], birds [52, 53] and musk shrew [54], and show high evolutionary conservation. However, no homologues have been found in rodents, thus preventing the generation of a knockout mouse as a model of the disease for examining pathogenetic mechanisms of KS in mammals.

The discovery of the KAL1 gene in the last decade has led to a series of studies on its distribution, biochemistry and functions.

The first expression studies carried out in chicken and human embryos showed that the gene is expressed in many structures of the central nervous system including the developing OBs and the cerebellum, but in different cell populations [55–57]. In particular, in chick embryos, a specific transcript was detected in the Purkinje cells of the cerebellum and in the mitral cell layer of the OBs. In humans, KAL1 gene expression was observed in layers of interneurons in OBs and cerebellum, and in the cerebral cortex [55, 56]. These expression studies were suggestive for a possible role of anosmin-1 in olfactory system development.

Later in 1999, Hardelin and co-authors [58] established that the KAL1-encoded protein, anosmin-1, is a restricted component of extracellular matrices transiently and regionally expressed during organogenesis in humans. Anosmin-1 was detected in the basement membranes and/or interstitial matrices of various structures including bronchial tubes, mesonephric tubules and duct, branches of the ureteric bud, muscular walls of the digestive tract and larger blood vessels, precartilaginous models of skeletal pieces, muscle tendons, head mesenchymes, inner ear and forebrain subregions. In the olfactory system, anosmin-1 was detected from week 5 onward, with a pattern of expression restricted to the OB presumptive region and, later, to the primitive OBs. In addition, at 6 weeks of gestation, anosmin-1 is present along the rostrocaudal migratory pathway of the GnRH-synthesizing neurons as well as in the medial walls of the primitive cerebral hemispheres.

The lack of a putative KAL1 homologous gene in rodents led to investigation of the presence of anosmin-1 by immunocytochemistry. In a study carried out using a series of antibodies raised against human anosmin-1, an immunoreactive signal in both mouse and rat embryos was found with a distribution of anosmin-1 similar to that found in chicken [59]. Again, anosmin-1 was clearly immunodetected in the developing olfactory epithelium, in the olfactory, vomeronasal and terminalis nerves, in the OBs, in the cerebellum and in the cerebral cortex, and in other brain areas of the musk shrew, a primitive eutherian mammal. In this model, GnRH neurons were seen in close association with anosmin-1 immunoreactive fibers [54]. This finding indicates that despite the difficulty to isolate the orthologous gene, anti-human anosmin-1 antibodies may recognize the anosmin-1 protein itself or some homologous molecules alternatively used in rodents.

Preliminary information about the physiology of anosmin-1 first came from *in vitro* studies carried out in COS [60] and CHO [61] cells. These studies showed that the X-KS gene product is a diffusible glycosylated protein with an approximate molecular mass of 100 kDa and that it is proteolytically processed on the cell membrane to yield a 45-kDa diffusible component that binds tightly to cell surfaces [60].

The predicted structure of anosmin-1 consists of different domains comprising an N-terminal signal peptide, followed by a cysteine-rich domain, a four-disulfide core domain typical of whey acidic proteins (WAPs), 4 fibronectin type III (FNIII) repeats and a C-terminal histidine-rich region (Fig. 2). The most highly conserved domains of anosmin-1 across species, from invertebrates to vertebrates, have been

found to be the WAP domain and the first FNIII domain, suggesting a crucial functional role of these regions.
WAPs are small proteins containing two four-disulfide core (4-DSC) domains, each comprising approximately 50 amino acids and including eight cysteine residues in a conserved arrangement [62]. The 4-DSC domains are not exclusive to the WAP proteins, with numerous other proteins encoding one or two of these domains. A large biological diversity exists between the proteins that contain one or two 4-DSC domains, with many being identified as protease inhibitors. Although the function of this domain in KAL1 protein is currently unknown, a possible anti-protease activity is intriguing since nerve growth cones express proteases on their surface to facilitate axonogenesis through the extracellular matrix [63, 64]. Protease inhibitors have been increasingly shown to play an important role in neuronal migration, by controlling degradation of matrix components, as demonstrated in the case of the serpin family [65]. In fact, Hu et al. [66] in a recent work showed that the serine protease urokinase-type plasminogen activator (UPA) may interact with anosmin-1, suggesting the hypothesis that anosmin-1 can modulate its catalytic activity.
On the other hand, the FNIII domains present in anosmin-1 are common motifs widely distributed amongst a large variety of proteins like extracellular matrix proteins (tenascin), cell adhesion molecules (i.e. NCAM, L1, TAG-1 and F3/contactin), receptor-linked protein kinases and tyrosine phosphatases [67–70]
Several of these molecules have been implicated in processes of neuronal migration and axonal targeting [71–73]. Interestingly, the FNIII repeats present in anosmin-1 are more similar to those found in the cellular adhesion molecules L1 [74], TAG-1 [75], and F3/contactin [69, 76]. L1 has been shown to be involved in X-linked hydrocephalus, another human neuronal migration defect [77, 78]. TAG-1 and F3/contactin are well-known examples of proteins mediating neurite outgrowth and reciprocal axonal interactions, displaying both adhesive and anti-adhesive properties. However, these molecules are membrane-bound proteins containing immunoglobulin C2 domains, unlike anosmin-1 which is a secreted extracellular protein.
Anosmin-1 has been shown to bind heparin, and its adhesive activity is dependent on cell surface heparan sulfate (HS) proteoglycans (HSPGs) [79]. In fact, the interaction of anosmin-1 with HS chains has been demonstrated recently by Hu et al. [66], who showed a high-binding affinity of anosmin-1 to HS using biochemical approaches. Furthermore, they found that HS might influence cell surface localization of the anosmin-1-uPA complex.
To study the physiological function of anosmin-1, many different biological approaches have been used. In *in vitro* studies, Soussi-Yanicostas and co-workers [79] showed that anosmin-1 serves as an adhesive substrate for different cell lines and that this adhesive property is dependent on the presence of HS and chondroitin sulfate glycosaminoglycans on the cell surfaces. Moreover, the same study found that such a substratum is permissive for neurite outgrowth of mouse cerebellar neurons. These effects are mediated by a conserved 32-amino-acid peptide located in the first FNIII repeat. In a more recent study carried out by the same group, anosmin-1 was found to promote axonal branch formation from OB output neurons, in mice and rats, suggesting the importance of this molecule in the formation and olfactory development of the LOT [59].
These results indicate that anosmin-1 is a branching and chemoattractive factor for LOT axons, leading to the hypothesis that the anosmia observed in KS patients could be due to the absence of OB in these patients, provided that the lack of LOT arborization leads to an involution of the OB. Moreover, the same paper, proposing a role for anosmin-1 in axon guidance events, supports the possibility that the neurological symptoms observed in KS patients could be due to axon guidance defects.
Using transgenic techniques in *C. elegans*, two separate groups showed a crucial role for anosmin-1 in axonal branching and epithelial morphogenesis [48, 49]. First, using three mutants that recapitulated X-KS mutations [11], Bülow et al. [48] have shown that in the nematode, WAP and FNIII domains appear to have different functions, with the WAP domain influencing axon targeting only, and the FNIII domains being essential for this function and for axonal branching.
CeKal-1 (the nematode homologue of KAL-1) is also required for ventral enclosure during embryogenesis and is first detected in a group of neuroblast descendants of the AB blastomere, which act as a substrate for epidermal migration following gastrulation [49]. Loss of *CeKal-1* function generates a number of striking phenotypes in mutants including ventral enclosure and ray (tail) abnormalities in males [49] and, although it is not clear whether *CeKal-1*-expressing neuroblasts provide an active signal or permissive substrate for migrating epithelial cells [80], vertebrate anosmin-1 can form adhesive contacts with mammalian epithelial cells [70, 79]. Significantly, *CeKal-1* mutants could also be rescued with the human or *C. elegans KAL1* sequences, indicating that the function of both anosmins are conserved [49]. In addition, the asymmetrical contacts between cells in *CeKal-1* mutants may provide a link to the abnormal kidney morphogenesis seen in a significant number of X-KS pedigrees.
A direct effect of anosmin-1 on GnRH neurons was not demonstrated until 2004, when we showed that anosmin-1 stimulates the *in vitro* chemomigration of immortalized GnRH neurons (see below). This was the first evidence that this protein may have a role in controlling migration of GnRH neurons in addition to events of axonal branching and morphogenesis [81].

The identification of disease-causing mutations frequently provides insight into protein function. More than 20 KAL1 mutations have been described and are believed to be loss-of-function mutations [for a complete list see ref. 82] (Fig. 2). These include stop and missense point mutations (most cases), intragenic deletions, complete gene deletion and even larger deletion of the Xp22.3 region associated with a contiguous gene syndrome (KS and ichthyosis) [36].

Some of the patients in whom point mutations were identified displayed, in addition to KS, mirror movements, pes cavus, high arched palate and unilateral renal aplasia, indicating that KAL plays a role in various developmental systems [83]. However, patients with KS who also manifest ichthyosis, chondrodysplasia punctata, mental retardation, short stature and ocular albinism are affected by a contiguous gene syndrome due to deletions of the distal short arm of the human X chromosome [84].

Very recently, seven new mutations for the KAL1 gene were identified: two amino acid substitutions were mapped in the first and fourth FNIII domains of anosmin-1. Notably, these mutations affect residues that are conserved between human and chicken proteins [57, 85]. Five KAL1 missense mutations, among a total of 25 different point mutations [82] had been reported in KAL patients, namely p.C163Y [86], p.C172R [11], p.N267K [83], p.E514K [87] and p.F517L [88]. The p.C163Y and p.C172R amino acid substitutions are located in the WAP-like domain of anosmin-1; both mutations are expected to disrupt disulfide bonds that are essential for the structure of this domain. The p.N267K, p.E514K, and p.F517L substitutions are located in structurally defined positions on the GFCC beta sheet of the first and third FNIII [89]. Molecular modeling and experimental studies predicted that two of these three mutations alter the basic surface charge of the protein, potentially interfering with binding to HS.

Bimanual synkinesia, bilateral cryptorchidism and unilateral renal agenesis were variably found in patients carrying these mutations. These findings underscored the functional importance of the WAP domain for GnRH and OB ontogeny, as well as in the corticospinal tract (responsible for mirror movements) and urogenital development (responsible for kidney agenesis).

Finally, anosmin-1 has also been proposed to play a role on development of the cribriform plate; in fact, the defect in olfactory axon elongation and GnRH neuron migration observed in KS could be due in part to an anomalous cribriform plate, and the observed alterations of the olfactory mucosa and nasal epithelia in some KS patients may support this hypothesis [90, 91]. However, in the only X-KS fetus studied so far, GnRH neurons and olfactory axons have clearly traversed the cribriform plate but they have remained trapped between the dura mater and the dorsal surface of the cribriform plate [28]. On the other hand, the craniofacial bone anomalies reported in autosomic KS due to KAL2 gene defects (see below) make this hypothesis much more attractive and worthy of further investigations.

The KAL2 gene encodes FGFR1

The KAL2 gene has been recently identified in KS patients with an autosomic dominant pedigree and it encodes FGFR1. Dode et al. [39] found overlapping interstitial deletions at 8p12-p11, a region that included the FGFR1 gene, in two individuals affected by contiguous gene syndromes. These authors also described heterozygous mutations in the 18 coding exons and splice sites of FGFR1 in both familial and sporadic cases of KS. Individuals affected by KS due to KAL2 mutations show typical HH and anosmia, but also other defects, such as agenesis of the corpus callosum, unilateral hearing loss, and fusion of the fourth and fifth metacarpal bones. In addition, cleft palate or lip and dental agenesis, two anomalies that are occasionally associated with KS [8, 92] are also present in individuals with mutations in FGFR1, suggesting their link with this form of the disease.

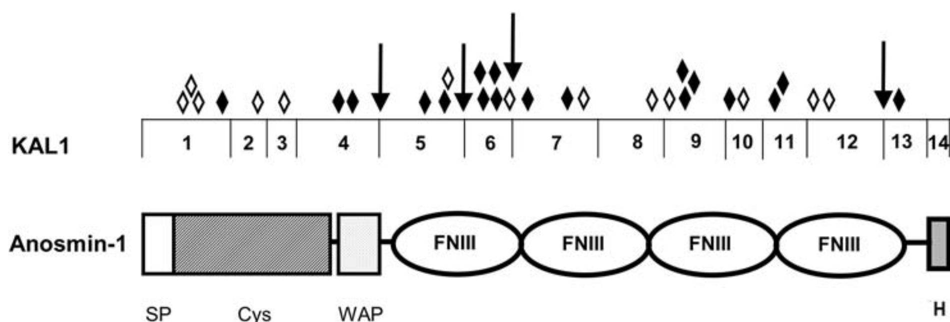

Figure 2. The schematic structure of the KAL1 gene, anosmin-1 and the location of the mutations found in KS. FNIII are fibronectin repeats of type III. SP, signal peptide; Cys, cysteine box; WAP, whey acidic protein-like domain; H, hystidine domain. Closed diamonds, point mutations; open diamonds, insertions or deletions; arrows, intron mutations. Exon and whole-gene deletions are not shown in the picture [from ref. 82].

Moreover, Sato and coworkers [41] found novel intragenic FGFR1 mutations in sporadic male cases of KS. The males with FGFR1 mutations had hypogonadotropic hypogonadism and anosmia but lacked other features. Finally, genomic breakpoints of a balanced reciprocal translocation t(7;8) (p12.3;p11.2) were also recently described in a male patient with HH and cleft lip and palate [93]. The translocation breaks up the FGFR1 sequence between exons 2 and 3, generating a novel fusion gene product. Although FGFR1 translocations producing fusion proteins have been reported as causes of myeloproliferative disorders, the finding of a constitutional FGFR1 translocation in KS associated with a developmental disorder is of particular interest. Even if these and other results indicate that haploinsufficiency for FGFR1 is a cause of cleft lip and palate [39, 41], the absence of detected anosmia in the patient with a translocation indicates that sufficient FGFR1 function may have been maintained to prevent the degree of agenesis of the olfactory lobes typically found in KS. FGFs make up a large family of polypeptide growth factors which are conserved in organisms ranging from nematodes to humans. There are at least 23 different members of the FGF family, all of them sharing a conserved core region of 120 amino acids. Members of the FGF family exert multiple roles during central nervous system development, and are potent modulators of cell proliferation, migration, differentiation and survival [94]. Several studies on the expression patterns of FGF ligands and receptors during central nervous system development as well as functional *in vitro* and *in vivo* assays have emphasized the critical role of FGFs in the initial generation of neural tissue at the stage of neural induction. This activity is also present in the rostral forebrain, directly affecting OB development (see below).

The FGFs produce their effects through transmembrane receptors, which are members of the tyrosine kinase family. Binding to FGFs and HSPGs leads to dimerization and subsequent activation of the receptor [see ref. 95 for a review]. Five FGF receptor genes, FGFR-1 to FGFR-5 have so far been identified; in addition, the presence of alternative splicing forms creates multiple receptor isoforms, with distinct specificities for particular FGFs.

The FGFR1 gene lies on chromosome 8, at 8p11.2-p11.1. The molecular structure of the protein is common to the other members of the FGF receptor family and consists of three immunoglobulin-like (Ig-like) domains, a stretch of acidic amino acids between Ig-like domains I and II, a heparin-binding domain in the extracellular binding region and an intracellular tyrosine kinase domain (Fig. 3) [96, 97]. Alternative tissue-specific splicing in Ig domain III dramatically changes the specificity of the FGFR for certain FGFs and is essential for directional FGF signaling. In contrast, another alternative splicing event, which eliminates Ig domain I, has little effect on ligand binding [98, 99]. *In vitro* assays have shown that FGFR1 isoforms have distinct binding affinities for the various FGFs, but only bind FGF1 and FGF2 with high affinity [100, 101]. Binding experiments demonstrated that multiple regions of the FGFR regulate ligand binding specificity and that one FGFR can interact with two molecules of FGF2. All this evidence suggests that the structural characteristics of the FGFR are important for the binding specificity of a large number of ligands and for the mechanism of activation by FGF and heparin/HS. In fact, a heparin/HSPG-binding site consisting of a stretch of 18 conserved residues (K18K) has been identified on the FGFR [97]; the K18K sequence is essential for receptor activity and by itself has the ability to interact with heparin. The identification of this site has suggested a model in which heparin could form a bridge between FGF and the FGFR.

FGF2 ligand and FGFR1 have been co-crystallized with heparin, and the structure of the complex defined [102]. Crystallization studies have also identified the location of an FGF-binding site in a region of the FGFR that includes Ig-like domains II and III and the linker domain [103]. There is also a potential heparin-binding cleft in the FGFR that incorporates the K18K peptide, which is contiguous with the heparin binding site on FGF2.

Notably, FGFR1 dimerization, induced by FGF binding, activates a variety of downstream signaling molecules by

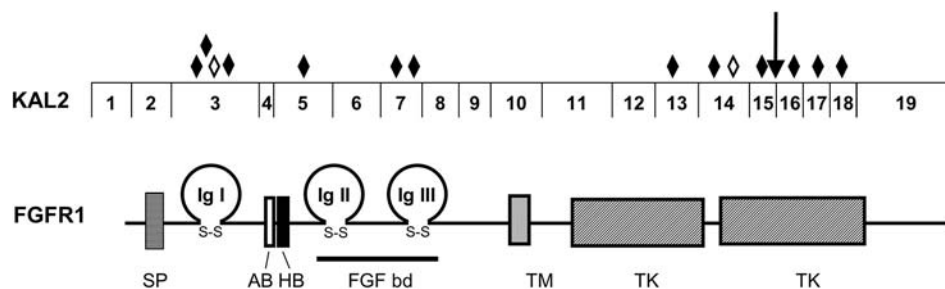

Figure 3. Schematic structure of KAL2 gene, fibroblast growth factor receptor 1 (FGFR1), and the location of the mutations found in KS. Ig I, Ig II and Ig III are the extracellular immunoglobin-like domains connected with disulfide bridges. The intracellular tyrosine kinase domain has two parts: a catalytic site and an ATP-binding site. SP, signal peptide; AB, acid box; HB, heparin-binding domain; FGF bd, FGF-binding domain; TM, transmembrane; TK, tyrosine kinase domain. Closed diamonds, point mutations; open diamonds, insertions or deletions; arrow, intron 15 mutation [data from ref. 82].

the autophosphorylation of tyrosine residues located in the intracellular region of the protein [95, 104]. The interaction of FGFs with their receptors may activate several downstream pathways. In particular, the signal transduction pathways that control central nervous system patterning and rostral forebrain development involve Six3 and Irx3 gene PLC-gamma- and Raf-dependent signaling [105, 106]. In addition, the small guanosine triphosphatases members, such as Rho, Rac, and Cdc42, known to control neuronal growth cones and cell migration and responses to chemoattractive/chemorepellent molecules, are also involved in FGF signal transduction [107, 108].
Several dysfunctions due to altered FGFR have been described. A constitutive gain-of-function mutation in the genes encoding FGFRs is responsible for the craniosynostosis present in Pfeiffer's syndrome (FGFR1 mutations) [109, 110], characterized by craniofacial anomalies, broad thumbs and big toes [111], and other skeletal dysplasia disorders, like the non-syndromic trigonocephaly, the Antley-Bixley syndrome, osteoglophonic dysplasia and in Jackson-Weiss syndrome [112–114, 115] However, no abnormality of the reproductive axis has been described in these syndromes.
In contrast, dominant forms of KS (KAL2) are linked to various loss-of-function mutations in FGFR1. At least 12 heterozygous nonsense or missense point mutations of FGFR1 have been so far characterized in familial and sporadic cases of the disease (Fig. 3) [82]. Moreover, small deletions leading to frameshift of the coding sequence and synthesis of truncated FGFR1 have also been reported. Several mutations substituted residues involved in receptor folding or in signal transduction [39, 41, 93] supporting the conclusion that autosomal KS may be due to inactivation of FGFR1.
The frontal mass problems, like dental agenesis and cleft palate, observed in KAL2 patients suggest a possible cranial neural crest defect. FGF was found to affect the patterning of both pre- and post-migratory cranial neural crest [116, 117], and this may explain the anomalies of many subjects affected by autosomal KS.
The observation that in subjects carrying KAL1 or KAL2 mutations the phenotype may be limited to anosmia suggests a role for FGFs in the development of the olfactory system [42] as well as a possible functional interaction between anosmin-1 and FGFR1-mediated effects [118].
Actually, several FGFs (i.e. FGF8, 3, 15, 17, and 18) are expressed in the developing telencephalon [119, 120]. In particular, FGF8 is expressed early in the mouse anterior forebrain and may play a key role in rostral telencephalic patterning [121]. Partial loss of function of the FGF8 gene during embryonic life leads to a small telencephalon, agenesis of OBs and an altered normal midline [122]. On the other hand, the initial contact between olfactory axons and the forebrain does occur in mice selectively lacking FGFR1 in the telencephalon. However, the OBs do not form normally from the neuroepithelial wall [42] and the animals show a defect of sense of smell. This defect in rostral telencephalon development may indirectly affect the migration of GnRH neurons (see below).
A functional interaction between KAL1 and KAL2 gene products has been proposed [39, 118]; in particular, the two factors have been suggested to converge on the control of a common downstream intracellular signaling [82]. As stated above, and in the previous section, both anosmin-1 and FGFs need the interaction with HSPG for their functions.
Recently, a functional interaction *in vitro* between anosmin-1 and the FGFR1-FGF2-HS complex has been reported. Such an interaction led to amplified responses in the FGFR1 signaling pathway that controls neurite outgrowth and cytoskeletal rearrangements of human embryonic olfactory neuroblasts [118].
Finally, anosmin-1 has also been proposed to interact directly with FGFR1 through its FNIII repeats; this is supported by the evidence that the two FNIII repeats of neural cell adhesion molecule (N-CAM) may bind to the extracellular region of this FGF receptor [123].
However, functional anosmin-1-FGF interaction in OB development needs further investigations to be confirmed.

Defect of GnRH neurons migration in Kallmann's disease

GnRH neurons comprise a small number of neuroendocrine neurons located in the hypothalamus of adult brain and are responsible for the secretion of the decapeptide GnRH. During development, they navigate an unusual pathway, migrating from their place of birth in the nasal compartment [124] to their final destinations scattered in the basal forebrain, in most vertebrates. Neuroendocrine GnRH neurons that regulate the pituitary-gonadal axis are usually referred as GnRH-1. In mammals, they appear to originate in the nasal region, in association with the medial wall of the olfactory placode, and migrate along the vomeronasal and the terminal nerves to gain access to the forebrain and to reach their final positions along the rostral-to-caudal continuum, extending from the medial septo/preoptic region of the forebrain to the posterior hypothalamus [26, 27, 125]. Even if a developmental association of GnRH neurons with the olfactory region has been assessed in several animal species (e.g. opossum, birds and fishes), the real origin of hypothalamic GnRH neurons is still debated [126]. The physical or genetic ablation of the olfactory placode in mice lead to a loss of hypothalamic GnRH neurons; in particular, the lack of the olfactory placode found in the mouse small-eye (Sey) homozygote mutant, which results from defect in the *Pax6* gene, correlates with the absence of GnRH neu-

rons in either the nose or the brain of the mouse at any embryonic age [127]. However, limitations of the olfactory placode ablation technique and the number of developmental defects found in Sey mouse (including defects in the migration of neural crest cells and in the development of anterior pituitary) do not conclusively support the placodal origin of GnRH neurons. [126]. Actually, new populations of forebrain GnRH neurons (called *neuromodulatory* GnRH neurons) have been identified; these neurons may originate from the anterior pituitary, from hypothalamic precursors as well as neural crest cells [124, 128–131], but they apparently do not contribute in the control of the reproductive axis. Once migrating neuroendocrine GnRH neurons have reached the hypothalamus, they project the axons to the median eminence, where they release GnRH into the pituitary portal vessels to induce the secretion of the gonadotropins from the anterior pituitary into the general circulation. These steps are key events for the development of a series of normal reproductive functions.

The mechanisms involved in the development of the migration route, as well as the molecular cues which control the movement of GnRH neurons along it, are not yet completely understood. In the last few years, a series of genes or proteins able to influence the migration of GnRH neurons *in vivo* and *in vitro* have been identified, [e.g. GABA, catecholamines, netrin-1, hepatocyte growth factor (HGF), growth arrest-specific gene 6 (Gas6), human nasal embryonic LHRH factor gene (NELF), stathmin, neural cell adhesion molecule and Ebf2; reviewed in refs. 132–135]. On the basis of their time of expression and their regional distribution, these factors have been suggested to act at different levels of the progression of the migratory process. In addition, some of these factors were found to affect indirectly the migration of GnRH neurons, i.e. by altering the development of the GnRH neurons themselves, or the components of the migratory route.

It must be underscored that, from the neurobiological point of view, a correct classification of factors specifically involved in the migration of GnRH neurons needs to be delineated in relation to the general criteria governing neuronal migration [136–139]. These include, for *in vitro* experiments (isolated cells, brain slices), the presence of specific receptors, the induction of random motility rather than that of directional chemotaxis (instructive vs permissive action), and the speed of migration (usually not less than 10 µm/h for neurons) [137]. On the other hand, for *in vivo* studies (i.e. like those performed on transgenic and knockout animals), it is important to assess whether the effects observed are cell autonomous (i.e. direct action on GnRH neurons) rather than due to a secondary action of the factors on the components of the migratory route (e.g. nasal mesenchyme, axons, vasculature or glial cells).

Unfortunately, the study of the GnRH-secreting neurons in animal models is generally hindered by their peculiar development and anatomical distribution. Several subpopulations of GnRH neurons have been described; in addition, they migrate during a developmentally narrow window of time (from embryonic day 11 to 18, in rodents) and these neurons are very limited in number and scattered into the septo/hypothalamic region in adulthood. The study of GnRH neurons has been facilitated by the development of cell lines of immortalized pure mouse GnRH-secreting neurons. Two different cell lines, GT1 cells (which include GT1-1, -3 and -7 subclones) [140] and GN cells (with GN10, GN11 and NLT subclones) [141] have been obtained by genetically targeted tumorigenesis of GnRH neurons in mice. Biochemical and immunological studies have shown that both cell lines express neuronal markers and retain the biological features of GnRH-secreting neurons. Moreover, a series of similarities between these immortalized neurons and normal GnRH neurons *in vivo* indicate that immortalized GnRH neurons can be considered an adequate model to study the biology of GnRH-secreting neurons [142]. It is interesting to underline that the GT1 and GN cell lines are representative, of well-differentiated post-migratory and of immature migratory GnRH neurons, respectively [143, 144]. From this perspective, we were the first to demonstrate, by various techniques and experimental paradigms (e.g. microchemotaxis assay, collagen gel invasion tests), that GN-derived clones (GN11 and NLT cells) retain a strong chemomigratory response *in vitro* to a series of stimuli such as fetal bovine serum, HGF, endothelin, vascular endothelial growth factor (VEGF) and Gas6 [142, 143, 145–148], suggesting their usefulness for the study of the multiple factors involved in the control of the migration of GnRH neurons in well-controlled culture conditions [134, 142].

In fact, the absence of a mouse model for the study of KS but the availability of such a cellular model of migrating GnRH neurons gave us the opportunity to study the role of anosmin-1 on purified mouse GnRH neurons. In particular, due to the ability of GN11 cells to migrate *in vitro* and to their characteristic phenotype of immature GnRH neurons, we decide to use them to test the possible chemotropic activity of anosmin-1.

By using this experimental paradigm, we were the first to show a direct action of anosmin-1 on the migratory activity of GnRH neurons. In fact, anosmin-1 produced a cell-specific chemotactic response of GN11 cells; moreover, none of the three forms of anosmin-1 carrying different missense mutations (N267K, E514K, F517L) found in patients affected by X-linked KS affected the chemomigration of GN11 cells.

We also found that anosmin-1 binds to the GN11 cell surface by interacting with the HSPG and the chemotactic effect of anosmin-1 can be specifically blocked by heparin

or heparinase pre-treatments [81]. In complete agreement with our findings, recent work done in fish, showed that the inactivation of the medaka KAL1 orthologue resulted in the disruption of forebrain GnRH neuronal migration [51], and that in zebrafish, the knockdown of *kal*1.1 gene function resulted in the loss of endocrine GnRH cells of the hypothalamus, but not of neuromodulatory GnRH cells of the midbrain and terminal nerve [128], thus suggesting once again the existence of separate pathways controlling the development of GnRH neurons and the possibility of a direct effect of anosmin-1 on GnRH neurons.

To correlate our *in vitro* results with the possible situation *in vivo*, we propose a role for anosmin-1 in attracting GnRH neurons to the forebrain. As stated above, anosmin-1 is a protein secreted into the extracellular matrix; anosmin-1 produced from the OB region could therefore diffuse in the adjacent extracellular matrix and create a concentration gradient, acting as a short-range chemoattractant for inward-migrating olfactory axons and GnRH neurons. Immunohistochemical studies carried out on human fetuses have shown that anosmin-1 is detected in the medial walls of the primitive cerebral hemispheres, on the migration path of the GnRH neurons and central roots of terminal nerves [58].

In summary, our results strongly support the evidence that anosmin-1 plays a key role in the control of migratory behavior of GnRH neurons, in addition to its known effect on olfactory nerve development, and provide an explanation for the infertility of patients affected by X-KS. In fact, before these findings, the more prevalent hypothesis for the HH observed in KS was that the defect of GnRH neuron migration was secondary to the absence of a proper scaffold, composed by the olfactory axons. However, a pleiotropic role for anosmin-1 on neuronal cell functions, which comprise axonal elongation and fasciculation as well as cell migration, cannot be excluded.

On the other hand, no direct evidence for a role of FGFR-1 on the migration of GnRH-secreting neurons has been clearly shown. Immunocytochemistry studies revealed the presence of FGFRs 1, 2 and 3, but not 4, in embryonic day (E) 10.5 medial nasal placode in mice. Dual immunocytochemistry confirmed the presence of FGFRs 1 and 3, but not 2 and 4, in a substantial fraction of E15.5 and postnatal day (P) 3 GnRH neurons. Moreover, the same paper shows that the addition of FGF2 to E15.5 and P3 GFP-GnRH neurons stimulated neurite outgrowth (E15.5 and P3) and branching (P3), suggesting a regulatory role of FGFs in GnRH axon targeting [149].

In addition, Gonzalez-Martinez and co-workers [118] have reported a functional interaction between anosmin-1 and the FGFR1-FGF2-HS complex, leading to amplified responses in the FGFR1 signaling pathway. In human embryonic GnRH olfactory neuroblasts, wild-type anosmin-1, but not proteins with loss-of-function KS mutations, induces neurite outgrowth and cytoskeletal rearrangements through FGFR1-dependent mechanisms involving a series of kinases. This study suggests that anosmin-1 is a co-ligand modulator of FGFR signaling that amplifies and specifies FGFR1 responses, thus providing a mechanism of linkage between autosomal and X-KS [118].

Concluding remarks

The reading of the human genome has opened up a new chapter in the physiopathology of many genetic diseases. Among them, KS continues to show a particularly prominent position. In fact, even if KS is not a lethal disease, it has raised the interest of many scientists for the specific features of the main systems involved (olfactory and reproductive) and for the biological defects included (endocrinological and neurobiological defects). Moreover, the cloning of two different genes (KAL1 and KAL2) responsible for certain forms of this complex disease has opened new perspectives to clarify general mechanisms governing brain development. Nevertheless, several conceptual issues still remain unsolved. For example, from a strictly experimental point of view, expression studies performed on human embryos have substantiated KAL1 expression in the developing OB, but with a cellular expression pattern different from the chicken (i.e. mitral cells compared with granular cell layer) [56]. However, since KAL1 is a secreted molecule, the significance of this discrepancy is still unclear.

From a pathogenetic view, the major critical point is the observation that hypogonadism and anosmia can occur together or separately within the same pedigree, suggesting that development of the OB may not always be linked or essential for GnRH development in humans. Moreover, subjects affected by X-KS without a clear hypogonadism and with normal-size testes have been described [10].

The possibility that abnormalities in KAL1 could cause such a distinct phenotype suggests that partial defects in GnRH migration and signaling may also occur and that other proteins could compensate for the genetic defect. Finally, the high phenotypic variability due to loss-of-function mutations of FGFR1 suggests, once again, that modifier genes or environmental factors are involved in determining the phenotype. It should also be recalled that patients with KS often display a variety of additional symptoms such as synkinesia, cerebellar abnormalities and unilateral renal aplasia. In humans, FGFs and their receptors are expressed in many embryo tissues; similarly, anosmin-1 is also expressed in several tissues outside the olfactory system, but the complete function of these factors in these tissues is still not fully understood.

Investigation of the mechanisms controlling GnRH neuronal migration might provide new insights into understanding the pathogenesis of KS. In recent years, several

factors involved in the migration of GnRH neurons have been characterized. Even though no mutations have been so far identified for most of the classical KS patients, these factors, or, alternatively, molecules that mediate their intracellular signaling, are potential candidates for the remaining 80% of familial and sporadic KS with unknown pathogenesis.

In a recent publication [151] the new p.W571R missense mutation has been described.

Acknowledgements. We would like to thank Dr. E. Rugarli (Laboratory of Physiopathology of Neurodegenerative Diseases, Division of Biochemistry and Genetics, Istituto Nazionale Neurologico, C. Besta IRCCS, Milan, Italy) for helpful discussions and comments on the manuscript.

1 Kallmann, F., Schoenfeld, W. and Barrere, S. (1944) The genetic aspects of primary eunuchoidism. Am. J. Mental Defic. 48, 203–236.
2 Jones, J. R. and Kemmann, E. (1976) Olfacto-genital dysplasia in the female. Obstet. Gynecol. Annu. 5, 443–466.
3 de Morsier, G. (1954) Etudes sur les dysraphies cranio-encephaliques. I. Agenesie des lobes olfactifs (telencephaloschizis lateral) et des commissures calleuse et anterieure (telencephaloschizis median). La dysplasie olfacto genitale. Schweiz. Arch. Neurol. Neurochir. Psychiat. 74, 309–361.
4 Naftolin, F., Harris, G. W. and Bobrow, M. (1971) Effect of purified luteinizing hormone releasing factor on normal and hypogonadotrophic anosmic men. Nature 232, 496–497.
5 Knorr, J. R., Ragland, R. L., Brown, R. S. and Gelber, N. (1993) Kallmann syndrome: MR findings. AJNR Am. J. Neuroradiol. 14, 845–851.
6 Madan, R., Sawlani, V., Gupta, S. and Phadke, R. V. (2004) MRI findings in Kallmann syndrome. Neurol. India. 52, 501–503.
7 Truwit, C. L., Barkovich, A. J., Grumbach, M. M. and Martini, J. J. (1993) MR imaging of Kallmann syndrome, a genetic disorder of neuronal migration affecting the olfactory and genital systems. AJNR Am. J. Neuroradiol. 14, 827–838.
8 White, B. J., Rogol, A. D., Brown, K. S., Lieblich, J. M. and Rosen, S. W. (1983) The syndrome of anosmia with hypogonadotropic hypogonadism: a genetic study of 18 new families and a review. Am. J. Med. Genet. 15, 417–435.
9 Wegenke, J. D., Uehling, D. T., Wear, J. B. Jr, Gordon, E. S., Bargman, J. G., Deacon, J. S., Herrmann, J. P. and Opitz, J. M. (1975) Familial Kallmann syndrome with unilateral renal aplasia. Clin. Genet. 7, 368–381.
10 Quinton, R., Duke, V. M., Robertson, A., Kirk, J. M., Matfin, G., de Zoysa, P. A., Azcona, C., MacColl, G. S., Jacobs, H. S., Conway, G. S., Besser, M., Stanhope, R. G. and Bouloux, P. M. (2001) Idiopathic gonadotrophin deficiency: genetic questions addressed through phenotypic characterization. Clin. Endocrinol. (Oxf.) 55, 163–174.
11 Oliveira, L. M., Seminara, S. B., Beranova, M., Hayes, F. J., Valkenburgh, S. B., Schipani, E., Costa, E. M., Latronico, A. C., Crowley, W. F.J. and Vallejo, M. (2001) The importance of autosomal genes in Kallmann syndrome: genotype-phenotype correlations and neuroendocrine characteristics. J. Clin. Endocrinol. Metab. 86, 1532–1538.
12 Burd, G. and Tolbert, L. (2000) Development of the olfactory system. In: Neurobiology of Taste and Smell (Finger, T., Silver, W. and Restrepo, D., Eds.), pp. 233–255. Wiley, Hoboken, N.J.
13 Mori, K., Nagao, H. and Yoshihara, Y. (1999) The olfactory bulb: coding and processing of odor molecule information. Science 286, 711–715.
14 Sullivan, S. L., Bohm, S., Ressler, K. J., Horowitz, L. F. and Buck, L. B. (1995) Target-independent pattern specification in the olfactory epithelium. Neuron 15, 779–789.
15 Graziadei, P. and Monti-Graziadei, A. (1992) The influence of the olfactory placode on the development of the telencephalon in *Xenopus laevis*. Neuroscience 46, 617–629.
16 Jiménez, D., Garcìa, C., de Castro, F., Chédotal, A., Sotelo, C., De Carlos, J., Valverde, F. and Lopez-Mascaraque, L. (2000) Evidence for intrinsic development of olfactory structures in Pax-6 mutant mice. J. Comp. Neurol. 428, 511–526.
17 Gong, Q. and Shipley, M. (1995) Evidence that pioneer olfactory axons regulate telencephalon cell cycle kinetics to induce the formation of the olfactory bulb. Neuron 14, 91–101.
18 Lopez-Mascaraque, L. and de Castro, F. (2002) The olfactory bulb as an independent developmental domain. Cell Death Differ. 9, 1279–1286.
19 Hinds, J. (1972) Early neuron differentiation in the mouse of olfactory bulb I. Electron Microsc. J. Comp. Neurol. 146, 253–276.
20 Farbman, A. and Squinto, L. (1985) Early development of olfactory receptor cell axons. Brain Res. 351, 205–213.
21 Tolbert, L., Oland, L., Tucker, E., Gibson, N., Higgins, M. and Lipscomb, B. (2004) Bidirectional influences between neurons and glial cells in the developing olfactory system. Prog. Neurobiol. 73, 73–105.
22 Lin, D. M. and Ngai, J. (1999) Development of the vertebrate main olfactory system. Curr. Opin. Neurobiol. 9, 74–78.
23 De Carlos, J. A., Lopez-Mascaraque, L. and Valverde, F. (1995) The telencephalic vesicles are innervated by olfactory placode-derived cells: a possible mechanism to induce neocortical development. Neuroscience 68, 1167–1178.
24 Doucette, J. R. (1984) The glial cells in the nerve fiber layer of the rat olfactory bulb. Anat. Rec. 210, 385–391.
25 Raisman, G. (1985) Specialized neuroglial arrangement may explain the capacity of vomeronasal axons to reinnervate central neurons. Neuroscience 14, 237–254.
26 Wray, S., Grant, P. and Gainer, H. (1989) 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.
27 Schwanzel-Fukuda, M. and Pfaff, D. W. (1989) Origin of luteinizing hormone-releasing hormone neurons. Nature 338, 161–165.
28 Schwanzel-Fukuda, M., Bick, D. and Pfaff, D. W. (1989) Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. Brain Res. Mol. Brain Res. 6, 311–326.
29 Hoffman, B. and Bradshaw, K. D. (2003) Delayed puberty and amenorrhea. Semin. Reprod. Med. 21, 353–362.
30 Nakagawa, K., Iwasaki, W., Sato, M., Ito, M., Kawachiya, S., Murashima, A., Kitagawa, M., Natori, M. and Saito, H. (2005) Successful pregnancy, achieved by ovulation induction using a human menopausal gonadotropin low-dose step-up protocol in an infertile patient with Kallmann's syndrome. J. Obstet. Gynaecol. Res. 31, 140–143.
31 Sato, N., Hasegawa, T., Hori, N., Fukami, M., Yoshimura, Y. and Ogata, T. (2005) Gonadotrophin therapy in Kallmann syndrome caused by heterozygous mutations of the gene for fibroblast growth factor receptor 1: report of three families: case report. Hum. Reprod. 20, 2173–2178.
32 Andria, G., Ballabio, A. and Parenti, G. (1987) X-linked ichthyosis due to steroid sulfatase deficiency associated with hypogonadism and anosmia. Ann. Neurol. 22, 98–99.
33 Lynch, H. T., Ozer, F., McNutt, C. W., Johnson, J. E. and Jampolsky, N. A. (1960) Secondary male hypogonadism and congenital ichthyosis: association of two rare genetic diseases. Am. J. Hum. Genet. 12, 440–447.

34 Perrin, J. C., Idemoto, J. Y., Sotos, J. F., Maurer, W. F. and Steinberg, A. G. (1976) X-linked syndrome of congenital ichthyosis, hypogonadism, mental retardation and anosmia. Birth Defects Orig. Artic. Ser. 12, 267–274.

35 Ballabio, A., Parenti, G., Tippett, P., Mondello, C., Di Maio, S., Tenore, A. and Andria, G. (1986) X-linked ichthyosis, due to steroid sulphatase deficiency, associated with Kallmann syndrome (hypogonadotropic hypogonadism and anosmia): linkage relationships with Xg and cloned DNA sequences from the distal short arm of the X chromosome. Hum. Genet. 72, 237–240.

36 Ballabio, A., Sebastio, G., Carrozzo, R., Parenti, G., Piccirillo, A., Persico, M. G. and Andria, G. (1987) Deletions of the steroid sulphatase gene in 'classical' X-linked ichthyosis and in X-linked ichthyosis associated with Kallmann syndrome. Hum. Genet. 77, 338–341.

37 Ballabio, A., Parenti, G., Carrozzo, R., Coppa, G., Felici, L., Migliori, V., Silengo, M., Franceschini, P. and Andria, G. (1988) X/Y translocation in a family with X-linked ichthyosis, chondrodysplasia punctata, and mental retardation: DNA analysis reveals deletion of the steroid sulphatase gene and translocation of its Y pseudogene. Clin. Genet. 34, 31–37.

38 Meitinger, T., Heye, B., Petit, C., Levilliers, J., Golla, A., Moraine, C., Dalla Piccola, B., Sippell, W. G., Murken, J. and Ballabio, A. (1990) Definitive localization of X-linked Kallman syndrome (hypogonadotropic hypogonadism and anosmia) to Xp22.3: close linkage to the hypervariable repeat sequence CRI-S232. Am. J. Hum. Genet. 47, 664–669.

39 Dode, C., Levilliers, J., Dupont, J. M., De Paepe, A., Le Du, N., Soussi-Yanicostas, N., Coimbra, R. S., Delmaghani, S., Compain-Nouaille, S., Baverel, F., Pecheux, C., Le Tessier, D., Cruaud, C., Delpech, M., Speleman, F., Vermeulen, S., Amalfitano, A., Bachelot, Y., Bouchard, P., Cabrol, S., Carel, J. C., Delemarre-van de Waal, H., Goulet-Salmon, B., Kottler, M. L., Richard, O., Sanchez-Franco, F., Saura, R., Young, J., Petit, C. and Hardelin, J. P. (2003) Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat. Genet. 33, 463–465.

40 Yazaki, N., Hosoi, Y., Kawabata, K., Miyake, A., Minami, M., Satoh, M., Ohta, M., Kawasaki, T. and Itoh, N. (1994) Differential expression patterns of mRNAs for members of the fibroblast growth factor receptor family, FGFR-1-FGFR-4, in rat brain. J. Neurosci. Res. 37, 445–452.

41 Sato, N., Katsumata, N., Kagami, M., Hasegawa, T., Hori, N., Kawakita, S., Minowada, S., Shimotsuka, A., Shishiba, Y., Yokozawa, M., Yasuda, T., Nagasaki, K., Hasegawa, D., Hasegawa, Y., Tachibana, K., Naiki, Y., Horikawa, R., Tanaka, T. and Ogata, T. (2004) Clinical assessment and mutation analysis of Kallmann syndrome 1 (KAL1) and fibroblast growth factor receptor 1 (FGFR1, or KAL2) in five families and 18 sporadic patients. J. Clin. Endocrinol. Metab. 89, 1079–1088.

42 Hebert, J. M., Lin, M., Partanen, J., Rossant, J. and McConnell, S. K. (2003) FGF signaling through FGFR1 is required for olfactory bulb morphogenesis. Development 130, 1101–1111.

43 Gannage-Yared, M. H., Dode, C., Ghanem, I., Chouery, E., Jalkh, N., Hardelin, J. P. and Megarbane, A. (2005) Coexistence of Kallmann syndrome and complete androgen insensitivity in the same patient. Eur. J. Endocrinol. 152, 813–817.

44 Abujbara, M. A., Hamamy, H. A., Jarrah, N. S., Shegem, N. S. and Ajlouni, K. M. (2004) Clinical and inheritance profiles of Kallmann syndrome in Jordan. Reprod. Health. 1, 5.

45 Franco, B., Guioli, S., Pragliola, A., Incerti, B., Bardoni, B., Tonlorenzi, R., Carrozzo, R., Maestrini, E., Pieretti, M., Taillon-Miller, P., Brown, C. J., Willard, H. F., Lawrence, C., Persico, M. G., Camerino, G. and Ballabio, A. (1991) A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature 353, 529–536.

46 Legouis, R., Hardelin, J. P., Levilliers, J., Claverie, J. M., Compain, S., Wunderle, V., Millasseau, P., Le Paslier, D., Cohen, D., Caterina, D. and et al. (1991) The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. Cell 67, 423–435.

47 Andrenacci, D., Le Bras, S., Rosaria Grimaldi, M., Rugarli, E. and Graziani, F. (2004) Embryonic expression pattern of the *Drosophila* Kallmann syndrome gene kal-1. Gene Expr. Patterns 5, 67–73.

48 Bulow, H. E., Berry, K. L., Topper, L. H., Peles, E. and Hobert, O. (2002) 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.

49 Rugarli, E. I., Di Schiavi, E., Hilliard, M. A., Arbucci, S., Ghezzi, C., Facciolli, A., Coppola, G., Ballabio, A. and Bazzicalupo, P. (2002) The Kallmann syndrome gene homolog in *C. elegans* is involved in epidermal morphogenesis and neurite branching. Development 129, 1283–1294.

50 Ardouin, O., Legouis, R., Fasano, L., David-Watine, B., Korn, H., Hardelin, J. and Petit, C. (2000) Characterization of the two zebrafish orthologues of the KAL-1 gene underlying X chromosome-linked Kallmann syndrome. Mech. Dev. 90, 89–94.

51 Okubo, K., Sakai, F., Lieng Lau, E., Yoshizaki, G., Takeuchi, Y., Naruse, K., Aida, K. and Nagahama, Y. (2005) Forebrain gonadotropin-releasing hormone neuronal development: insights from transgenic medaka and the relevance to X-linked Kallmann syndrome. Endocrinology 147, 1076–1084.

52 Legouis, R., Lievre, C. A., Leibovici, M., Lapointe, F. and Petit, C. (1993) Expression of the KAL gene in multiple neuronal sites during chicken development. Proc. Natl. Acad. Sci. USA 90, 2461–2465.

53 Rugarli, E. I. and Ballabio, A. (1993) Kallmann syndrome: from genetics to neurobiology. JAMA 270, 2713–2716.

54 Dellovade, T., Hardelin, J., Soussi-Yanicostas, N., Pfaff, D., Schwanzel-Fukuda, M. and Petit, C. (2003) Anosmin-1 immunoreactivity during embryogenesis in a primitive eutherian mammal. Brain Res. Dev. Brain Res. 140, 157–167.

55 Duke, V. M., Winyard, P. J., Thorogood, P., Soothill, P., Bouloux, P. M. and Woolf, A. S. (1995) KAL, a gene mutated in Kallmann's syndrome, is expressed in the first trimester of human development. Mol. Cell Endocrinol. 110, 73–79.

56 Lutz, B., Kuratani, S., Rugarli, E. I., Wawersik, S., Wong, C., Bieber, F. R., Ballabio, A. and Eichele, G. (1994) Expression of the Kallmann syndrome gene in human fetal brain and in the manipulated chick embryo. Hum. Mol. Genet. 3, 1717–1723.

57 Rugarli, E. I., Lutz, B., Kuratani, S. C., Wawersik, S., Borsani, G., Ballabio, A. and Eichele, G. (1993) Expression pattern of the Kallmann syndrome gene in the olfactory system suggests a role in neuronal targeting. Nat. Genet. 4, 19–26.

58 Hardelin, J. P., Julliard, A. K., Moniot, B., Soussi-Yanicostas, N., Verney, C., Schwanzel-Fukuda, M., Ayer-Le Lievre, C. and Petit, C. (1999) 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.

59 Soussi-Yanicostas, N., de Castro, F., Julliard, A. K., Perfettini, I., Chedotal, A. and Petit, C. (2002) Anosmin-1, defective in the X-linked form of Kallmann syndrome, promotes axonal branch formation from olfactory bulb output neurons. Cell 109, 217–228.

60 Rugarli, E. I., Ghezzi, C., Valsecchi, V. and Ballabio, A. (1996) 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.

61 Soussi-Yanicostas, N., Hardelin, J. P., Arroyo-Jimenez, M. M., Ardouin, O., Legouis, R., Levilliers, J., Traincard, F., Betton,

J. M., Cabanie, L. and Petit, C. (1996) 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.
62 McKnight, R. A., Burdon, T., Pursel, V. G., Shamay, A., Wall, R. J. and Hennighausen, L. (1992) The whey acidic protein. Cancer Treat. Res. 61, 399–412.
63 Letourneau, P. C., Condic, M. L. and Snow, D. M. (1992) Extracellular matrix and neurite outgrowth. Curr. Opin. Genet. Dev. 2, 625–634.
64 McGuire, P. G. and Seeds, N. W. (1990) Degradation of underlying extracellular matrix by sensory neurons during neurite outgrowth. Neuron 4, 633–42.
65 Osterwalder, T., Contartese, J., Stoeckli, E. T., Kuhn, T. B. and Sonderegger, P. (1996) Neuroserpin, an axonally secreted serine protease inhibitor. EMBO J. 15, 2944–2953.
66 Hu, Y., Gonzalez-Martinez, D., Kim, S. H. and Bouloux, P. M. (2004) 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.
67 Lander, A. (1989) Understanding the molecules of neural cell contacts: emerging patterns of structure and function. Trends Neurosci. 12, 189–195.
68 Edelman, G. and Crossin, K. (1991) Cell adhesion molecules: implications for a molecular histology. Annu. Rev. Biochem. 60, 155–190.
69 Gennarini, G., Cibelli, G., Rougon, G., Mattei, M. and Goridis, C. (1989) The mouse neuronal cell surface protein F3: a phosphatidylinositol-anchored member of the immunoglobulin superfamily related to chicken contactin. J. Cell Biol. 109, 775–788.
70 Robertson, A., MacColl, G. S., Nash, J. A., Boehm, M. K., Perkins, S. J. and Bouloux, P. M. (2001) Molecular modelling and experimental studies of mutation and cell-adhesion sites in the fibronectin type III and whey acidic protein domains of human anosmin-1. Biochem. J. 357, 647–659.
71 Dodd, J. and Jessell, T. M. (1988) Axon guidance and the patterning of neuronal projections in vertebrates. Science 242, 692–699.
72 Reichardt, L. F. and Tomaselli, K. J. (1991) Extracellular matrix molecules and their receptors: functions in neural development. Annu. Rev. Neurosci. 14, 531–570.
73 Chiquet-Ehrismann, R., Matsuoka, Y., Hofer, U., Spring, J., Bernasconi, C. and Chiquet, M. (1991) Tenascin variants: differential binding to fibronectin and distinct distribution in cell cultures and tissues. Cell Regul. 2, 927–938.
74 Moos, M., Tacke, R., Scherer, H., Teplow, D., Fruh, K. and Schachner, M. (1988) Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature 334, 701–703.
75 Felsenfeld, D. P., Hynes, M. A., Skoler, K. M., Furley, A. J. and Jessell, T. M. (1994) TAG-1 can mediate homophilic binding, but neurite outgrowth on TAG-1 requires an L1-like molecule and beta 1 integrins. Neuron 12, 675–690.
76 Ranscht, B. (1988) Sequence of contactin, a 130-kD glycoprotein concentrated in areas of interneuronal contact, defines a new member of the immunoglobulin supergene family in the nervous system. J. Cell Biol. 107, 1561–1573.
77 Rosenthal, A., Jouet, M. and Kenwrick, S. (1992) Aberrant splicing of neural cell adhesion molecule L1 mRNA in a family with X-linked hydrocephalus. Nat. Genet. 2, 107–112.
78 Van Camp, G., Vits, L., Coucke, P., Lyonnet, S., Schrander-Stumpel, C., Darby, J., Holden, J., Munnich, A. and Willems, P. J. (1993) A duplication in the L1CAM gene associated with X-linked hydrocephalus. Nat. Genet. 4, 421–425.
79 Soussi-Yanicostas, N., Faivre-Sarrailh, C., Hardelin, J. P., Levilliers, J., Rougon, G. and Petit, C. (1998) Anosmin-1 underlying the X chromosome-linked Kallmann syndrome is an adhesion molecule that can modulate neurite growth in a cell-type specific manner. J. Cell Sci. 111, 2953–2965.
80 Chin-Sang, I. and Chisholm, A. (2000) Form of the worm: genetics of epidermal morphogenesis in C. elegans. Trends Genet. 16, 544–551.
81 Cariboni, A., Pimpinelli, F., Colamarino, S., Zaninetti, R., Piccolella, M., Rumio, C., Piva, F., Rugarli, E. I. and Maggi, R. (2004) 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.
82 Dode, C. and Hardelin, J. P. (2004) Kallmann syndrome: fibroblast growth factor signaling insufficiency? J. Mol. Med. 82, 725–734.
83 Hardelin, J. P., Levilliers, J., Blanchard, S., Carel, J. C., Leutenegger, M., Pinard-Bertelletto, J. P., Bouloux, P. and Petit, C. (1993) Heterogeneity in the mutations responsible for X chromosome-linked Kallmann syndrome. Hum. Mol. Genet. 2, 373–377.
84 Ballabio, A. and Andria, G. (1992) Deletions and translocations involving the distal short arm of the human X chromosome: review and hypotheses. Hum. Mol. Genet. 1, 221–227.
85 Legouis, R., Cohen-Salmon, M., del Castillo, I., Levilliers, J., Capy, L., Mornow, J. P. and Petit, C. (1993) Characterization of the chicken and quail homologues of the human gene responsible for the X-linked Kallmann syndrome. Genomics 17, 516–518.
86 Sato, N., Katsumata, N., Kagami, M., Hasegawa, T., Hori, N., Kawakita, S., Minowada, S., Shimotsuka, A., Shishiba, Y., Yokozawa, M., Yasuda, T., K., N., D., H., Hasegawa, Y., Tachibana, K., Naiki, Y., Horikawa, R., Tanaka, T. and Ogata, T. (2004) Clinical assessment and mutation analysis of Kallmann syndrome 1 (KAL1) and fibroblast growth factor receptor 1 (FGFR1, or KAL2) in five families and 18 sporadic patients. J. Clin. Endocrinol. Metab. 89, 1079–1088.
87 Maya-Nunez, G., Cuevas-Covarrubias, S., Zenteno, J. C., Ulloa-Aguirre, A., Kofman-Alfaro, S. and Mendez, J. P. (1998) Contiguous gene syndrome due to deletion of the first three exons of the Kallmann gene and complete deletion of the steroid sulphatase gene. Clin. Endocrinol. (Oxf.) 48, 713–718.
88 Georgopoulos, N. A., Pralong, F. P., Seidman, C. E., Seidman, J. G., Crowley, W. F. Jr and Vallejo, M. (1997) Genetic heterogeneity evidenced by low incidence of KAL-1 gene mutations in sporadic cases of gonadotropin-releasing hormone deficiency. J. Clin. Endocrinol. Metab. 82, 213–217.
89 Robertson, A., MacColl, G. S., Nash, J. A., Boehm, M. K., Perkins, S. J. and Bouloux, P. M. (2001) Molecular modelling and experimental studies of mutation and cell-adhesion sites in the fibronectin type III and whey acidic protein domains of human anosmin-1. Biochem. J. 357, 647–659.
90 Quinton, R., Hasan, W., Grant, W., Thrasivoulou, C., Quiney, R. E., Besser, G. M. and Bouloux, P. M. (1997) Gonadotropin-releasing hormone immunoreactivity in the nasal epithelia of adults with Kallmann's syndrome and isolated hypogonadotropic hypogonadism and in the early midtrimester human fetus. J. Clin. Endocrinol. Metab. 82, 309–314.
91 Fuerxer, F., Carlier, R., Iffenecker, C., Schaison, G. and Doyon, D. (1996) Magnetic resonance imaging of the olfactory pathways in Kallmann de Morsier syndrome. J. Neuroradiol. 23, 223–230.
92 Hardelin, J. P. (2001) Kallmann syndrome: towards molecular pathogenesis. Mol. Cell Endocrinol. 179, 75–81.
93 Kim, H., Herrick, S., Lemyre, E., Kishikawa, S., Salisz, J., Seminara, S., MacDonald, M., Bruns, G., Morton, C., Quade, B. and Gusella, J. (2005) Hypogonadotropic hypogonadism and cleft lip and palate caused by a balanced translocation producing haploinsufficiency for FGFR1. J. Med. Genet. 42, 666–672.

94 Ford-Perriss, M., Abud, H. and Murphy, M. (2001) Fibroblast growth factors in the developing central nervous system. Clin. Exp. Pharmacol. Physiol. 28, 493–503.
95 Eswarakumar, V. P., Lax, I. and Schlessinger, J. (2005) Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev. 16, 139–149.
96 Johnson, D. and Williams, L. (1993) Structural and functional diversity in the FGF receptor multigene family. Adv. Cancer Res. 60, 1–41.
97 Kan, M., Wang, F., Xu, J., Crabb, J., Hou, J. and McKeehan, W. (1993) An essential heparin binding domain in the fibroblast growth factor receptor kinase. Science 259, 1918–1921.
98 Wang, F., Kan, M., Yan, G., Xu, J. and McKeehan, W. (1995) Alternately spliced NH2-terminal immunoglobulin-like loop I in the ectodomain of the fibroblast growth factor (FGF) receptor 1 lowers affinity for both heparin and FGF-1. J. Biol. Chem. 270, 10231–10235.
99 Duan, D.-S., Werner, S. and Williams, L. (1992) A naturally occurring secreted form of fibroblast growth factor (FGF) receptor 1 binds basic FGF in preference over acidic FGF. J. Biol. Chem. 267, 16076–16080.
100 Xu, J., Liu, Z. and Ornitz, D. (2000) Temporal and spatial gradients of Fgf8 and Fgf17 regulate proliferation and differentiation of midline cerebellar structures. Development 127, 1833–1843.
101 Ornitz, D. and Itoh, N. (2001) Fibroblast growth factors. Genome Biol. 2, 3005.1–3005.12.
102 Schlessinger, J., Plotnikov, A., Ibrahimi, O., Eliseenkova, A., BK, Y., Yayon, A., RJ, L. and Mohammadi, M. (2000) Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGFR binding and dimerization. Mol. Cell 6, 743–750.
103 Plotnikov, A., Schlessinger, J., Hubbard, S. and Mohammadi, M. (1999) Structural basis for FGF receptor dimerization and activation. Cell Death Differ. 98, 641–650.
104 Groth, C. and Lardelli, M. (2002) The structure and function of vertebrate fibroblast growth factor receptor 1. Int. J. Dev. Biol. 46, 393–400.
105 Kobayashi, D., Kobayashi, M., Matsumoto, K., Ogura, T., Nakafuku, M. and Shimamura, K. (2002) Early subdivisions in the neural plate define distinct competence for inductive signals. Development 129, 83–93.
106 Umbhauer, M., Penzo-Mendez, A., Clavilier, L., Boucaut, J. and Riou, J. (2000) Signaling specificities of fibroblast growth factor receptors in early *Xenopus* embryo. J. Cell Sci. 113, 2865–2875.
107 Hall, A. (1998) Rho GTPases and the actin cytoskeleton. Science 279, 509–514.
108 Nikolic, M. (2002) The role of Rho GTPases and associated kinases in regulating neurite outgrowth. Int. J. Biochem. Cell Biol. 34, 731–745.
109 Rutland, P., Pulleyn, L., Reardon, W., Baraitser, M., Hayward, R., Jones, B., Malcolm, S., Winter, R., Oldridge, M., Slaney, S., Poole, M. and Wilkie, A. (1995) Identical mutations in the FGFR2 gene cause both Pfeiffer and Crouzon syndrome phenotypes. Nat. Genet. 9, 173–176.
110 Muenke, M., Schell, U., Hehr, A., Robin, N., Losken, H., Schinzel, A., Pulleyn, L., Rutland, P., Reardon, W., Malcolm, S. and Winter, R. (1994) A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. Nat. Genet. 8, 269–274.
111 Bellus, G., Gaudenz, K., Zackai, E., Clarke, L., Szabo, J., Francomano, C. and Muenke, M. (1996) Identical mutations in three different fibroblast growth factor receptor genes in autosomal dominant craniosynostosis syndromes. Nat. Genet. 14, 174–176.
112 Kress, W., Petersen, B., Collmann, H. and Grimm, T. (2000) An unusual FGFR1 mutation (fibroblast growth factor receptor 1 mutation) in a girl with non-syndromic trigonocephaly. Cytogenet. Cell Genet. 91, 138–140.
113 Hurley, M., White, M., Green, A. and Kelleher, J. (2004) Antley-Bixler syndrome with radioulnar synostosis. Pediatr. Radiol. 34, 148–151.
114 White, K., Cabral, J., Davis, S., Fishburn, T., Evans, W., Ichikawa, S., Fields, J., Yu, X., Shaw, N., McLellan, N., McKeown, C., FitzPatrick, D., Yu, K., Ornitz, D. and Econs, M. (2005) Mutations that cause osteoglophonic dysplasia define novel roles for FGFR1 in bone elongation. Am. J. Hum. Genet. 76, 361–367.
115 Roscioli, T., Flanagan, S., Kumar, P., Masel, J., Gattas, M., Hyland, V. and Glass, I. (2000) Clinical findings in a patient with FGFR1 P252R mutation and comparison with the literature. Am. J. Med. Genet. 93, 22–28.
116 Morriss-Kay, G. and Wilkie, A. (2005) Growth of the normal skull vault and its alteration in craniosynostosis: insights from human genetics and experimental studies. J. Anat. 207, 637–653.
117 Sarkar, S., Petiot, A., Copp, A., Ferretti, P. and Thorogood, P. (2001) FGF2 promotes skeletogenic differentiation of cranial neural crest cells. Development 128, 2143–2152.
118 Gonzalez-Martinez, D., Hu, Y. and Bouloux, P. M. (2004) Ontogeny of GnRH and olfactory neuronal systems in man: novel insights from the investigation of inherited forms of Kallmann's syndrome. Front. Neuroendocrinol. 25, 108–130.
119 Maruoka, Y., Ohbayashi, N., Hoshikawa, M., Itoh, N., Hogan, B. L. and Furuta, Y. (1998) Comparison of the expression of three highly related genes, Fgf8, Fgf17 and Fgf18, in the mouse embryo. Mech. Dev. 74, 175–177.
120 Shinya, M., Koshida, S., Sawada, A., Kuroiwa, A. and Takeda, H. (2001) Fgf signalling through MAPK cascade is required for development of the subpallial telencephalon in zebrafish embryos. Development 128, 4153–4164.
121 Crossley, P. H. and Martin, G. R. (1995) The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. Development 121, 439–451.
122 Meyers, E. N., Lewandoski, M. and Martin, G. R. (1998) An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. Nat. Genet. 18, 136–141.
123 Kiselyov, V. V., Skladchikova, G., Hinsby, A. M., Jensen, P. H., Kulahin, N., Soroka, V., Pedersen, N., Tsetlin, V., Poulsen, F. M., Berezin, V. and Bock, E. (2003) Structural basis for a direct interaction between FGFR1 and NCAM and evidence for a regulatory role of ATP. Structure (Camb.) 11, 691–701.
124 el Amraoui, A. and Dubois, P. (1993) Experimental evidence for an early commitment of gonadotropin-releasing hormone neurons, with special regard to their origin from the ectoderm of nasal cavity presumptive territory. Neuroendocrinology 57, 991–1002.
125 Kim, K., Patel, L., Tobet, S., King, J., Rubin, B. and Stopa, E. (1999) Gonadotropin-releasing hormone immunoreactivity in the adult and fetal human olfactory system. Brain Res. 826, 220–229.
126 Whitlock, K. (2005) Origin and development of GnRH neurons. Trends Endocrinol. Metab. 16, 145–151.
127 Dellovade, T., Pfaff, D. and Schwanzel-Fukuda, M. (1998) The gonadotropin-releasing hormone system does not develop in Sey mouse phenotype. Dev. Brain Res. 107, 233–240.
128 Whitlock, K., Smith, K., Kim, H. and Harden, M. (2005) A role for foxd3 and sox10 in the differentiation of gonadotropin-releasing hormone (GnRH) cells in the zebrafish *Danio rerio*. Development 132, 5491–5502.
129 Whitlock, K., Wolf, C. and Boyce, M. (2003) Gonadotropin-releasing hormone (GnRH) cells arise from cranial neural crest and adenohypophyseal regions of the neural plate in the zebrafish, *Danio rerio*. Dev. Biol. 257, 140–152.

130 Quanbeck, C., Sherwood, N., Millar, R. and Terasawa, E. (1997) Two populations of luteinizing hormone-releasing hormone neurons in the forebrain of the rhesus macaque during embryonic development. J. Comp. Neurol. 380, 293–309.
131 Daikoku, S. and Koide, I. (1998) Spatiotemporal appearance of developing LHRH neurons in the rat brain. J. Comp. Neurol. 393, 34–47.
132 MacColl, G., Bouloux, P. and Quinton, R. (2002) Kallmann syndrome: adhesion, afferents, and anosmia. Neuron 34, 675–678.
133 Wierman, M. E., Pawlowski, J. E., Allen, M. P., Xu, M., Linseman, D. A. and Nielsen-Preiss, S. (2004) Molecular mechanisms of gonadotropin-releasing hormone neuronal migration. Trends Endocrinol. Metab. 15, 96–102.
134 Maggi, R., Cariboni, A., Zaninetti, R., Samara, A., Stossi, F., Pimpinelli, F., Giacobini, P., Consalez, G. G., Rugarli, E. and Piva, F. (2005) Factors involved in the migration of neuroendocrine hypothalamic neurons. Arch. Ital. Biol. 143, 171–178.
135 Tobet, S. and Schwarting, G. (2006) Recent progress in gonadotropin-releasing hormone neuronal migration. Endocrinology 147, 1159–1165.
136 Rakic, P. (1990) Principles of neural cell migration. Experientia 46, 882–891.
137 Fishman, R. B. and Hatten, M. E. (1993) Multiple receptor systems promote CNS neural migration. J. Neurosci. 13, 3485–3495.
138 Nadarajah, B., Brunstrom, J. E., Grutzendler, J., Wong, R. O. L. and Pearlman, A. L. (2001) Two modes of radial migration in early development of the cerebral cortex. Nat. Neurosci., 143–150.
139 Marin, O. and Rubenstein, J. L. (2001) A long, remarkable journey: tangential migration in the telencephalon. Nat. Rev. Neurosci. 2, 780–790.
140 Mellon, P. L., Windle, J. J., Goldsmith, P. C., Padula, C. A., Roberts, J. L. and Weiner, R. I. (1990) Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. Neuron 5, 1–10.
141 Radovick, S., Wray, S., Lee, E., Nicols, D. K., Nakayama, Y., Weintraub, B. D., Westphal, H., Cutler, G. B.J. and Wondisford, F. E. (1991) Migratory arrest of gonadotropin-releasing hormone neurons in transgenic mice. Proc. Natl. Acad. Sci. USA 88, 3402–3406.
142 Pimpinelli, F. and Maggi, R. (2004) Immortalised neurons as a model to study the signals involved in the migration of gonadotropin-releasing hormone (GnRH) neurons: basic and clinical implications. Recent Res. Dev. Endocrinol. 4, 143–146.
143 Pimpinelli, F., Redaelli, E., Restano-Cassulini, R., Curia, G., Giacobini, P., Cariboni, A., Wanke, E., Bondiolotti, G. P., Piva, F. and Maggi, R. (2003) Depolarization differentially affects the secretory and migratory properties of two cell lines of immortalized luteinizing hormone-releasing hormone (LHRH) neurons. Eur. J. Neurosci. 18, 1410–1418.
144 Prioni, S., Loberto, N., Prinetti, A., Chigorno, V., Guzzi, F., Maggi, R., Parenti, M and Sonnino, S. (2003) Sphingolipid metabolism and caveolin expression in gonadotropin-releasing hormone-expressing GN11 and gonadotropin-releasing hormone-secreting GT1–7 neuronal cells. Neurochem. Res. 27, 831–840.
145 Maggi, R., Pimpinelli, F., Molteni, L., Milani, M., Martini, L. and Piva, F. (2000) Immortalized luteinizing hormone-releasing hormone (LHRH) neurons show a different migratory activity '*in vitro*'. Endocrinology 141, 2105–2112.
146 Giacobini, P., Giampietro, C., Fioretto, M., Maggi, R., Cariboni, A., Perroteau, I. and A., F. (2002) Hepatocyte growth factor/scatter factor facilitates migration of GN-11 immortalized LHRH neurons. Endocrinology 143, 3306–3315.
147 Allen, M. P., Linseman, D. A., Udo, H., Xu, M., Schaack, J. B., Varnum, B., Kandel, E. R., Heidenreich, K. A. and Wierman, M. E. (2002) Novel mechanism for gonadotropin-releasing hormone neuronal migration involving Gas6/Ark signaling to p38 mitogen-activated protein kinase. Mol. Cell Biol. 22, 599–613.
148 Romanelli, R., Barni, T., Maggi, M., Luconi, M., Failli, P., Pezzatini, A., Morelli, A., Maggi, R., Zaninetti, R., Salerno, R., Ambrosini, S., Marini, M., Rotella, C. and GB, V. (2005) Role of endothelin-1 in the migration of human olfactory gonadotropin-releasing hormone-secreting neuroblasts. Endocrinology 146, 4321–4330.
149 Gill, J. C., Moenter, S. M. and Tsai, P. S. (2004) Developmental regulation of gonadotropin-releasing hormone neurons by fibroblast growth factor signaling. Endocrinology 145, 3830–3839.
150 Rugarli, E. I. (1999) Kallmann syndrome and the link between olfactory and reproductive development. Am. J. Hum. Genet. 65, 943–948.
151 Albuisson, J., Pecheux, C., Carel, J. C., Lacombe, D., Leheup, B., Lapuzina, P., Bouchard, P., Legius, E., Matthijs, G., Wasniewska, M., Delpech, M., Young, J., Hardelin, J. P. and Dode, C. (2005) Kallman syndrome: 14 novel mutations in KAL1 and FGFR1 (KAL2). Hum. Mutat. 25, 98–99.

To access this journal online:
http://www.birkhauser.ch