Septo-Optic Dysplasia: From Mouse to Man

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Abstract. Septo-optic dysplasia (SOD) is a highly variable condition which is characterized by midline neurological abnormalities associated with pituitary hypoplasia and optic nerve hypoplasia. Recently, a novel homeobox gene, *Hesx1*, has been implicated in this condition. The gene encodes a 185-aminoacid transcription factor and is expressed early at gastrulation in the mouse embryo. It is initially expressed in prospective forebrain tissue, but later becomes restricted to Rathke's pouch, the primordium of the anterior pituitary gland. Null mutant mice lacking *Hesx1* exhibit a phenotype comprising variable anterior CNS defects such as a reduced prosencephalon, abnormalities in the corpus callosum and the septum pellucidum, anophthalmia or microphthalmia, defective olfactory development and bifurcations in Rathke's pouch with pituitary dysplasia. Since this phenotype strikingly resembled the phenotype of septo-optic dysplasia, we cloned and sequenced the human homologue *HESX1* and screened for mutations in affected individuals using single-stranded conformational polymorphism analysis (SSCP). Two siblings with SOD from a highly consanguineous family were homozygous for an Arg53Cys missense mutation within the *HESX1* homeodomain. This led to a loss of *in vitro* DNA binding. Subsequently, we have identified heterozygous mutations in *HESX1* which are associated with milder pituitary phenotypes, suggesting an autosomal dominant inheritance with variable penetrance. Hence, our studies indicate a vital role for *Hesx1/HESX1* in forebrain and pituitary development in mouse and man.

Key words: SOD, pituitary, homeobox, *HESX1*, transcription factors

Introduction

Homeobox genes play a crucial role in regional patterning and cell differentiation during the development of multicellular organisms. They were first described in *Drosophila* and are characterized by the presence of a homeobox which encodes a highly conserved 60-amino acid homeodomain. Protein products of these genes can therefore act as specific transcription factors

which bind to regulatory elements of target genes by means of the homeodomain. The homeobox gene family can be subdivided into about 30 classes of genes, each characterized by its own specific consensus sequence of the homeodomain (1). Mutations in several homeobox genes have been identified as the molecular defects in several human anomalies, including *PAX2* (renal-coloboma syndrome), *PAX3* (Waardenberg syndrome type I), *PAX6* (aniridia), *MSX1* (selective tooth agenesis), *MSX2* (craniosynostosis), *HOXD13* (limb deformities), *PIT1* and *PROP1* (hypopituitarism and combined hormone deficiencies) and *SHOX* (short stature) (2–13).

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The role of homeobox genes in development is elegantly demonstrated by the pituitary gland, which will be discussed in greater detail in this article. The pituitary gland consists of the anterior, intermediate and posterior lobes. The embryonic origin of the anterior and intermediate lobes is distinct from that of the posterior lobe, which is derived from neural ectoderm at the base of the developing diencephalon.

Development of the Anterior Pituitary Gland

The anterior pituitary gland arises as a midline structure from the anterior neural ridge immediately anterior to the adjacent region of the neural plate from which the hypothalamus and posterior pituitary will develop (14, 15). A number of transcription factors, which are homeodomain proteins encoded by homeobox genes, are expressed in the area of the pituitary primordium. These include *Six3* (16), *Pax6* (17) and *Hesx1/Rpx* (18, 19), all of which continue to be expressed in Rathke's pouch, which first appears as an invagination of oral ectoderm derived from the most anterior ectoderm of the early embryo. In the mouse, the anterior and intermediate lobes of the pituitary gland form at embryonic day 8.5 (E8.5) from Rathke's pouch, which makes direct cell-cell contact with the neuroepithelium of the nascent diencephalon at E8.5–9 (20). Concurrent with organ commitment, two LIM-homeodomain factors, *Lhx3* or *P-Lim* (21, 22) and *Lhx4* (23), are also expressed in Rathke's pouch. Deletion of the *Lhx3/P-Lim* gene in mice results in the failure of the pituitary gland to grow and differentiate, although Rathke's pouch forms (24). However, a double *Lhx3/Lhx4* mutant results in a failure of formation of Rathke's pouch (23). Mutations of *LHX3* in man result in a highly specific phenotype characterized by panhypopituitarism which is a consequence of severe anterior pituitary hypoplasia and a short neck which is associated with disturbed muscular function leading to a significant limitation of head rotation (25). The

OTX-related factors *Ptx1/P-OTX* and *Ptx2* are also expressed throughout early pituitary development (26–28).

The mature anterior pituitary gland is populated by five neuroendocrine cell types, each defined by the hormone produced: corticotropes (ACTH), thyrotropes (TSH), gonadotropes (LH, FSH), somatotropes (GH) and lactotropes (PRL). Each of these cell types differentiates in a temporally and spatially regulated manner (29, 30), and this process is dependent upon a number of transcription factors. A nuclear receptor, SF1, is expressed later in the nascent gonadotrope lineage (31). A tissue-specific POU domain factor Pit1 is required for terminal differentiation, growth and survival of somatotropes, lactotropes and thyrotropes (32). Additionally, a paired-like homeodomain factor Prop1 is also required for the determination of the Pit1-dependent lineage (33). Mutations of both *Pit1* and *Prop1* have been described in man and account for the phenotype of GH, prolactin and TSH deficiency (10, 11) and GH, prolactin, TSH and FSH/LH deficiency (12) respectively. In addition to these intrinsic factors, the development of the anterior pituitary gland is dependent on various extrinsic factors. The apposition of Rathke's pouch and the diencephalon is maintained throughout the early stages of pituitary organogenesis, and this close relationship together with various animal experimental manipulations has long suggested that inductive tissue interactions are involved in the process. A role for genes such as *Ttf1*, *BMP4* and *Fgf8* (34–36), which are expressed in the diencephalon and not Rathke's pouch, has been suggested for normal pituitary development.

The Homeobox Gene *Hesx1*

Hesx1 is a member of the paired-like class of homeobox genes (18) and is first expressed during mouse embryogenesis in a small patch of cells in the anterior midline visceral endoderm as gastrulation commences (19, 37). Expression is

then induced in the adjacent ectoderm in an area destined to give rise to the ventral prosencephalon, which will in turn form the forebrain (38). This ectodermal expression appears to require an inductive signal from the visceral endoderm (37). Subsequently, *Hesx1* is expressed at the anterior extreme of the rostral neural folds, finally resolving to the ventral diencephalon by E9.0 days. At this stage, *Hesx1* is also expressed in the thickened layer of oral ectoderm which will give rise to Rathke's pouch, the primordium of the anterior pituitary. *Hesx1* continues to be expressed in the developing anterior pituitary until E12 days when its transcripts disappear in a spatiotemporal sequence which corresponds to progressive pituitary cell differentiation. Expression is undetectable by E16 days.

In the Ames dwarf mouse, the phenotype of which arises as a result of a mutation in the *Prop1* gene, *Hesx1* expression persists until E16 days, suggesting a role for *Prop1* in regulating *Hesx1* expression (33). Additionally, a role for *Lhx3* in the maintenance of *Hesx1* expression has been suggested by the *Lhx3* null mutants (24). Since *Hesx1* is a putative repressor gene (39), its role in pituitary development may be to repress downstream target genes.

Targeted disruption of *Hesx1* in the mouse revealed morphological defects in homozygote embryos from E9 days and, also, in mutant neonates and adults (39). These included a reduction in prospective forebrain tissue, absence of developing optic vesicles, markedly decreased head size, craniofacial dysplasia with a short nose and severe microphthalmia. The defects were often asymmetrical, with one side affected more than the other. By E10 days, the severity of the mutant phenotype was variable, and mutants could be divided into either a Class I phenotype which was severe or a milder Class II phenotype. All homozygotes were abnormal at birth, as were 1% of the heterozygotes. Histological analysis of the mutant mice revealed that Class I embryos at E13 days had substantially reduced forebrains with no

sign of telencephalic vesicle or infundibulum development. Additionally, absence of the optic cups, the olfactory placodes and Rathke's pouch was noted. Class II mutant embryos demonstrated a thin forebrain epithelium with retardation in prosencephalic development. Other abnormalities included reduced telencephalic vesicles with hypothalamic abnormalities and aberrant morphogenesis of Rathke's pouch. The olfactory pits and optic vesicles were also hypoplastic and often asymmetric in appearance.

Further analysis of neonatal and adult mutants revealed hypoplastic nasal cavities, hypoplastic olfactory bulbs, microphthalmia and anophthalmia. Abnormalities of the septum pellucidum and corpus callosum were also noted. The variable forebrain midline defects associated with abnormal morphogenesis of the anterior pituitary gland in the mutant mice were reminiscent of the syndrome of septo-optic dysplasia (SOD) in man (39).

Septo-Optic Dysplasia (SOD)

This is a rare condition which was initially described by Reeves (40) in a seven month old baby, and is often referred to as de Morsier syndrome (41). It is a highly heterogeneous condition defined loosely by any combination of optic nerve hypoplasia, midline neuroradiological abnormalities such as agenesis of the corpus callosum and absence of the septum pellucidum, and pituitary hypoplasia with consequent panhypopituitarism (40–46). The phenotype is highly variable, and may include midline defects without optic nerve hypoplasia. Pituitary hypoplasia may manifest as endocrine deficits varying from isolated growth hormone deficiency to panhypopituitarism. Either sexual precocity or failure to develop in puberty may occur, and abnormal hypothalamic neuroanatomy or function may be a feature (47, 48), as may diabetes insipidus (49).

Several aetiologies have been postulated to

account for the sporadic occurrence of SOD, such as viral infections, environmental teratogens, and vascular or degenerative damage (48, 50). Familial cases of SOD are rare, and may be associated with an autosomal recessive inheritance (51–53). In the light of the phenotype demonstrated in *Hesx1* null mutant mice, we investigated the role of the human homologue of this gene in patients with SOD. We isolated the human homologue *HESX1* from a human genomic library. The gene was cloned and sequenced and mapped to chromosome 3p21.1–3p21.2. The *HESX1* locus spans 1.7 kB with a highly conserved genomic organization consisting of four coding exons. The 185-amino acid open reading frame is highly conserved compared with the mouse and *Xenopus* open reading frames, particularly across the homeodomains which share 95% and 80% homology respectively. An additional conserved domain is located near the amino-terminus, which is also present in other homeodomain classes including *engrailed* and *gooseoid* and has been postulated to play a role in transcriptional repression.

We have now screened over one hundred patients with a variety of pituitary disorders. Southern blot analysis performed on 38 of these patients revealed no deletions or gross rearrangements associated with the phenotype. Our patient population included a highly consanguineous family in which two affected siblings presented with optic nerve hypoplasia, absence of the corpus callosum, and hypoplasia of the pituitary gland with panhypopituitarism (51). SSCP analysis using primers flanking each of the four *HESX1* coding exons was used to screen these patients for point mutations within the *HESX1* ORF. We found a homozygous mis-sense mutation in the homeobox of *HESX1* in the 2 affected siblings which resulted in the substitution of a highly conserved arginine residue (Arg53) by cysteine. The parents were heterozygous for the mutation. This mutation led to the loss of a *Cac8I* restriction site, and this was used to screen for the

mutated allele within the extended family. This confirmed that the affected siblings were homozygous for the mutation and revealed 9 phenotypically normal heterozygotes within this highly consanguineous pedigree, consistent with an autosomal recessive inheritance. Loss of the *Cac8I* restriction site was not observed in 10 unrelated patients with sporadic SOD or pituitary hypoplasia, nor in 70 normal individuals.

The Arg53 residue is one of the most highly conserved residues in homeodomain proteins from a broad range of eukaryotic species. It has been shown to contact the DNA phosphate backbone in the paired- and engrailed-DNA crystal structures (54, 55). A non-conservative substitution of this amino-acid by cysteine would be expected to compromise DNA binding. To test this, recombinant wild-type and mutant HESX1 proteins were purified and their DNA binding activity assayed in an electromobility shift assay, using the palindromic PIII sequence to which murine *Hesx1* has previously been shown to bind (33). Wild-type HESX1 binds this sequence as a dimer with a high affinity even at concentrations of 1nM, whereas the mutant protein fails to bind even at micromolar concentrations (39). The severe reduction in *in vitro* PIII binding activity of the mutant HESX1 protein compared with the wild type protein suggests that the Arg53Cys substitution may act as a null mutation *in vivo*.

We have subsequently found further heterozygous mutations/polymorphisms of *HESX1* in children with SOD and are currently investigating the functional significance of these. These will shed further light on the function of various domains of the *HESX1* protein, and the role of the gene in human forebrain and pituitary development.

Conclusions

Mutations in homeobox genes have been shown to play a role in the pathogenesis of a number of conditions in man. We have

demonstrated a role for the homeobox gene *Hesx1* in a rare familial form of SOD. We are currently investigating the role of the gene in the more frequently occurring sporadic form of SOD. Given that a mild phenotype was observed in 1% of *Hesx1* heterozygous mutants, it remains possible that some de novo heterozygous mutations may be responsible for milder cases of SOD. Additionally, SOD may have a multigenic basis, to which alterations in *HESX1* could contribute. Alterations in regulatory regions may also contribute to the aetiology of this condition. Whatever the mechanism, the striking resemblance between the mouse and human phenotype suggests that the gene may play a similar role in human and mouse embryonic development.

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