SOX2 Haploinsufficiency is Associated with Slow Progressing Hypothalamo-Pituitary Tumours

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ABSTRACT: SOX2 is an early developmental transcription factor and marker of stem cells that has recently been implicated in the development of the pituitary gland. Heterozygous SOX2 mutations have been described in patients with hypopituitarism and severe ocular abnormalities. In the majority of published cases, the pituitary gland is either small or normal in size. Here, we report two unrelated patients with SOX2 haploinsufficiency (a heterozygous gene deletion and a novel c.143TC>AA/p.F48X mutation) who developed nonprogressive pituitary tumours of early onset, suggesting a congenital etiology. The truncating mutation resulted in significant loss of function and impaired nuclear localization of the mutant protein, in addition to a failure to repress β-catenin transcriptional activity in vitro. This is the first indication that SOX2 haploinsufficiency is implicated in the generation of pituitary tumours with distinct clinical characteristics, possibly mediated via its effects on the Wnt signalling pathway.

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SOX2 (MIM# 184429), a member of the SOXB1 family of transcription factors, is a widely expressed marker of progenitor and stem cells. The single exon gene encodes a 317 amino acid protein that contains an N-terminal domain, a DNA-binding high-mobility group (HMG) domain and a C-terminal transcriptional activation domain [Collignon et al., 1996]. SOX2 haploinsufficiency in both mouse and human has been associated with variable hypopituitarism associated with anterior pituitary hypoplasia, suggesting that it has a critical role in the development of the anterior pituitary [Kelberman et al., 2006]. Heterozygous de novo mutations in humans are associated with severe ocular phenotypes (bilateral anophthalmia or severe microphthalmia) and hypogonadotropic hypogonadism (HH) with or without associated abnormalities such as esophageal atresia, male genital anomalies, developmental delay, sensorineural deafness, hippocampal malformation, hypoplasia of the corpus callosum, and hypothalamic hamartoma [Kelberman et al., 2006, 2008]. In humans, SOX2 expression is detected within Rathke’s pouch and maintained throughout the development of the anterior pituitary, as well as in the presumptive hypothalamus and neural ectoderm [Kelberman et al., 2008]. In neural progenitors, SOX2 downregulation is associated with progression from a proliferating undifferentiated state to a committed phenotype [Graham et al., 2003]. In the murine adult pituitary, SOX2 expression is maintained in a small population of cells lining the pituitary cleft, which show many of the properties of progenitor cells and have the ability to differentiate into all hormone-producing cell types [Fauquier et al., 2008].

The specificity of the action of SOX proteins depends largely on their interaction with partner proteins. Recent data have suggested an interaction with components of another early developmental pathway, the Wnt/β-catenin signaling pathway. Members of the Wnt/β-catenin pathway are expressed in the developing pituitary and are implicated in the maintenance of normal morphology of the gland and the determination of hormone-secreting cell types [Olson et al., 2006; Potok et al., 2008]. Xenopus XSox3 and XSox17 as well as murine SOX2 interact with β-catenin and repress its activity in vitro [Mansukhani et al., 2005; Zorn et al., 1999]. We have shown that human SOX2 is also capable of inhibiting β-catenin-mediated transcriptional activation [Kelberman et al., 2008]. Recent studies have suggested that aberrant activation of the Wnt pathway, resulting from sustained β-catenin activation or downregulation of Wnt-inhibitors is associated with the development of pituitary tumors [Buslei et al., 2005; Elston et al., 2008; Gaston-Massuet et al., 2011]. Here we report, for the first time to our knowledge, the identification of heterozygous SOX2 mutations in two unrelated patients in association with pituitary tumours of likely congenital origin and we provide in vitro evidence that disruption of the SOX2/β-catenin interaction may be the molecular mechanism underlying some human pituitary tumours.

Case 1 is a female patient with bilateral anophthalmia who presented for the first time at the age of 18 years for assessment of
pubertal delay. She was the second child of nonconsanguineous parents born at term with a birth weight of −1.0 standard deviation score (SDS), and had severely impaired language development and delayed motor milestones. At presentation, she was prepubertal (Tanner staging 1) with a height of 144.8 cm (−3.12 SDS). Basal endocrine investigations demonstrated undetectable estradiol with low basal gonadotropins and a flat luteinising hormone (LH) and follicle stimulating hormone (FSH) response to GnRH stimulation confirming a diagnosis of HH (Table 1). Magnetic resonance imaging (MRI) revealed a sellar tumor with a cystic component, consistent with HH (1.73 ± 0.36 SD) activation of the basal reporter activity (t-test, P = 0.0006). Similar effects have been reported for other SOX2 mutant proteins previously identified in patients with hypopituitarism and eye defects [Kelberman et al., 2006, 2008].

Finally, we investigated the ability of the p.F48X mutant protein to antagonise the β-catenin-mediated activation of the TOPFLASH reporter [Korinek et al., 1997] (Fig. 1I). Transfection of the constitutively active p.S33Y mutant β-catenin resulted in an 89.7-fold (±4.6 SD) activation of the basal TOPFLASH reporter activity. This transactivation of the reporter was significantly reduced when cells were cotransfected with plasmids expressing wild-type SOX2 (2.8-fold, ±0.34 SD, t-test, P < 0.0001). In contrast, cotransfection of a construct expressing the p.F48X mutant SOX2 failed to suppress the β-catenin-mediated activation of TOPFLASH compared with the wild-type SOX2 (72.3-fold, ±4.91 SD, t-test, P = 0.0002). These differences were not due to impaired synthesis of SOX2 protein, as differences were not due to impaired synthesis of SOX2 protein, as differences were not due to impaired synthesis of SOX2 protein, as differences were not due to impaired synthesis of SOX2 protein, as differences were not due to impaired synthesis of SOX2 protein, as differences were not due to impaired synthesis of SOX2 protein, as differences were not due to impaired synthesis of SOX2 protein, as differences were not due to impaired synthesis of SOX2 protein.
Identification and characterization of novel SOX2 heterozygous mutations in association with slow-growing sellar tumors. (A and B) Midline sagittal (left) and coronal (right) MRI scans of patient 1 at 18 years (A) and 28 years of age (B) revealing a large mass in the location of the pituitary gland (arrows). (C) Array-CGH profile from patient 1 showing the ratio of probes, each represented by a single dot plotted as a function of chromosome position; loss of copy number of a probe shifts the ratio to the left showing deletion of SOX2. (D and E) Midline sagittal (left) and coronal (right) MRI scans of patient 2 at 17 months (D) and at 32 months of age (E) showing a large mass in the location of the pituitary gland (arrows). (F) Sequence electropherogram showing a two-base substitution c.143_144TT>AA (p.F48X) in patient 2. Nucleotide numbering reflects cDNA with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. (G) Overexpression of FLAG-tagged SOX2 p.F48X in HEK293T cells results in impaired localization of the protein whereas FLAG-tagged wild type SOX2 localizes in the nucleus. Nuclei are counterstained with DAPI. (H) Luciferase assay using the Hexx1 minimal promoter demonstrates impaired activation of the promoter through expression of SOX2 p.F48X (t-test, P = 0.0006). (I) Luciferase assay using the TOPFLASH reporter reveals that expression of the SOX2 p.F48X mutant protein does not result in repression of Wnt signaling, activated by expression of a stable mutant form of β-catenin (p.S33Y) (t-test, P = 0.0002), whereas expression of wild-type SOX2 represses this activation (t-test, P < 0.0001). (J) Western blot analysis showing similar expression levels of both wild-type (WT) and p.F48X proteins. Although the intensity of the signal obtained with the α-FLAG antibody is not comparable, this is due to unequal loading of protein extracts in the gel, as shown by the loading control (α-β-Actin).
as in small cell lung cancer [Maddison et al., 2010], squamous head and neck carcinomas [Freier et al., 2010], meningiomas [Comtesse et al., 2005], glioblastomas [Schmitz et al., 2007], pancreatic [Sanada et al., 2006], hepatocellular and bladder carcinomas, prostate cancer, and in seminomas [Schoenhals et al., 2009]. These findings are in contrast to our report whereby SOX2 haploinsufficiency, rather than its upregulation, resulted in the development of a pituitary mass, which would suggest that SOX2 can also act as a tumor suppressor. Indeed, the oncogenic/tumor suppressor potential of SOX2 seems to be cell-type dependent, SOX2 expression is downregulated in gastric carcinomas [Li et al., 2004; Otsubo et al., 2008] and in regions of intestinal metaplasia in patients with Barrett’s esophagus. In addition, the esophagus of hypomorphic mutant mice (Sox2<sup>−/−</sup>), expressing only 17% of wild-type SOX2 levels, has an appearance resembling mucus metaplasia [Qte et al., 2007]. In these cell types, as in the developing pituitary, SOX2 may play a role in the control of the cell cycle through its interaction with the canonical Wnt signaling pathway. Wnt signaling plays an essential role during pituitary development in the control of proliferation of Rathke’s pouch precursors and in the differentiation of the Pouf1-lineage (also known as Ptt1) [Olson et al., 2006]. Overactivation of the Wnt pathway in the mouse leads to hyperplasia of the embryonic pituitary due to a significant increase in proliferation [Gaston-Massuet et al., 2008]. Moreover, overactivating mutations in β-catenin have been detected in up to 90% of adamantinomatous craniopharyngiomas (ACP) in humans [Buslei et al., 2005; Kato et al., 2004], a benign and slow-growing hypothalmo-pituitary tumor, while Wnt inhibitors are downregulated in other pituitary tumors [Elston et al., 2008]. We have recently shown that mice expressing a degradation-resistant mutant β-catenin in Rathke’s pouch develop pituitary tumors that closely resemble human ACP [Gaston-Massuet et al., 2011]. Our luciferase data clearly show that the p.F48X mutant protein cannot repress β-catenin-mediated transcriptional activation, which is compatible with a model whereby the two identified patients may have developed a pituitary mass during development due to increased proliferation. The nonprogressive nature of these otherwise impressive pituitary lesions supports the suggestion that they have an embryological origin. It could be postulated that in these cases failure of SOX2 to repress β-catenin activity may not be sufficient to sustain the growth of the pituitary mass and other factors (including other members of the SOX family) may be compensating for the impaired SOX2 function.

It is intriguing that only the two patients described in this study, out of many known patients with SOX2 haploinsufficiency, developed pituitary tumors. Suprasellar lesions have so far been reported in six patients with SOX2 mutations, including three cases of hypothalamic hamartomas [Kelberman et al., 2006; Scheider et al., 2009] and three cases of suprasellar arachnoid cysts [Kelberman et al., 2008; Scheider et al., 2009; Wang et al., 2008]. SOX2 mutations are associated with considerable variability in phenotypes and apart from the severe ocular defects, patients rarely exhibit the full spectrum of clinical manifestations. The sensitivity of different tissues to altered dosage of SOX2 may in part explain this observation. As illustrated in the two cases reported here, it is possible that for some congenital pituitary tumors careful follow-up, rather than surgical excision, may be the treatment of choice.

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References

Bass AJ, Watanabe H, Merkel CH, Yu S, Perner S, Verhaak RG, Kim SY, Wardwell L, Tamayo P, Gat-Viks I, Ramos AH, Woo MS, Weir BA, Getz G, Beroukhim R, O’Keeley M, Dutt A, Rozenblatt-Rosen O, Dziunyev P, Komisarof J, Chirieac LR, Lafaigre CJ, Scheble V, Wilbertz T, Ma C, Rao S, Nakagawa H, Stairs DB, Lin L, Gerendai TJ, Wagner P, Minna JD, Gazdar AF, Zhu CQ, Breeze MS, Cseccelion L, Jr UR, Marie SK, Dahl O, Shivdasani RA, Tsao MS, Rubin MA, Wong KK, Regev A, Hahn WC, Beer DG, Rustgi AK, Meyerson M. 2009. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 41:1238–1242.

Buslei R, Nolde M, Hofmann B, Messner S, Fypouglov YJ, Siebzhuhoff P, Hahnen E, Krischniuk G, Falhbusch R. 2005. Common mutations of beta-catenin in adamantinomatous craniopharyngiomas but not in other tumours originating from the sellar region. Acta Neuropathol 109:589–597.

Chen Y, Shi L, Zhang L, Li R, Liang J, Yu W, Sun L, Yang X, Wang Y, Zhang Y, Shang Y. 2008. The molecular mechanism governing the oncogenic potential of SOX2 in breast cancer. J Biol Chem 283:17965–17978.

Collignon J, Scockanathan S, Hacker A, Cohen-Tannoudji M, Norris D, Rastan S, Stevanovic M, Goodfellow PN, Lovell-Badge R. 1996. A comparison of the properties of Sox-3 with Sry and two related genes, Sox-1 and Sox-2. Development 120:509–520.

Comtesse N, Zippel A, Walle S, Monz D, Backes C, Fischer U, Mayer J, Ludwig N, Hildebrandt A, Keller A, Steudel W, Lenhofer HP, Meese E. 2005. Complex humoral immune response against a benign tumor: frequent antibody response against specific antigens as diagnostic targets. Proc Natl Acad Sci USA 102:9601–9606.

Elston MS, Gill AJ, Conaglen JV, Clarkson A, Shaw JM, Law AJ, Cook RJ, Little NS, Clifton-Bligh RJ, Robinson BG, McDonald KL. 2008. Wnt pathway inhibitors are strongly down-regulated in pituitary tumours. Endocrinology 149:1235–1242.

Eroshkin F, Kazanskaya O, Martyanova N, Zaraisky A. 2002. Characterization of cis-regulatory elements of the homeobox gene Xant-1. Gene 285:279–286.

Fauquier T, Rizzotti K, Dattani M, Lovell-Badge R, Robinson IC. 2008. SOX2-expressing progenitor cells generate all of the major cell types in the adult mouse pituitary gland. Proc Natl Acad Sci USA 105:2907–2912.

Freier K, Knoepfe K, Flechtenmacher C, Pungs S, Devens F, Toedt G, Hofele C, Joos S, Lichter P, Radwimmer B. 2010. Recurrent copy number gain of transcription factor SOX2 and corresponding high protein expression in oral squamous cell carcinoma. Genes Chrom Cen 49:9–16.

Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kexraye R, Vernay B, Jacques TS, Takedo MM, Le Tissier P, Dattani MT, Martinez-Barbera JP. 2011. Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumours in mice and humans. Proc Natl Acad Sci USA 12:11482–11487.

Gaston-Massuet C, Andoniadou CL, Signore M, Sajedi E, Bird S, Turner JM, Martinez-Barbera JP. 2008. Genetic interaction between the homeobox transcription factors HESX1 and SIX3 is required for normal pituitary development. Dev Biol 324:322–333.

Graham V, Khudyakov J, Ellis P, Pevny L. 2003. SOX2 functions to maintain neural progenitor identity. Neuron 39:749–765.

Kato K, Nakatani Y, Kamto H, Inayama Y, Ittii R, Nagahara H, Miyake T, Tanaka M, Ito Y, Aida N, Tachibana K, Sekido K, Tanaka Y. 2004. Possible linkage between specific histological structures and aberrant reactivation of the Wnt pathway in adamantinomatous craniopharyngioma. J Pathol 205:814–821.

Kelberman D, de Castro SC, Huang S, Crolla JA, Palmer R, Gregory JW, Taylor D, Cavalllo L, Fienza FM, Fischetto R, Achermann JC, Martinez-Barbera JP, Rizzotti K, Lovell-Badge R, Robinson JC, Cerrelli D, Dattani MT. 2008. SOX2 plays a critical role in the pituitary, forebrain, and eye during human embryonic development. J Clin Endocrinol Metab 93:1865–1873.

Kelberman D, Rizzotti K, Avilov A, Bitner-Glindzicz M, Gianfani S, Collins J, Chong WK, Kirk JM, Achermann JC, Ross R, Carmignac D, Lovell-Badge R, Robinson IC, Dattani MT. 2006. Mutations within Sox-2/SOX2 are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. J Clin Invest 112:2442–2455.

Korinek V, Barker N, Morin PJ, van Wulfften Palthe CH, Li X, Akiyama Y, Tani M, Takizawa T, Koike M, Yuasa Y. 2000. Expression of the SRY-related HMG box protein SOX2 in human gastric gland. Proc Natl Acad Sci USA 107:2907–2912.

Li XL, Eishi Y, Bai YQ, Sakai H, Akiyama Y, Tani M, Takizawa T, Koike M, Yuasa Y. 2004. Expression of the SV40-related HMG box protein SOX2 in human gastric carcinoma. Int J Oncol 24:257–263.

Maddison P, Thorpe A, Silcocks P, Robertson JF, Chapman CJ. 2010. Autoimmunity to SOX2, clinical phenotype and survival in patients with small-cell lung cancer. Lung Cancer 70:335–339.

Manoukian A, Ambrosetti D, Holmes G, Cornivelii L, Basilico C. 2005. Sox2 induction by FGF and FGF2 activating mutations inhibits Wnt signaling and osteoblast differentiation. J Cell Biol 168:1065–1076.
Olson LE, Tollkuhn J, Scafoglio C, Krones A, Zhang J, Obi KA, Wu W, Taketo MM, Klemper R, Grosschedl R, Rose D, Li X, Rosenfeld MG. 2006. Homeodomain-mediated beta-catenin-dependent switching events dictate cell-lineage determination. Cell 125:593–605.

Otsubo T, Akiyama Y, Yanagihara K, Yuasa Y. 2008. SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. Br J Cancer 98:824–831.

Potok MA, Cha KB, Hunt A, Brinkmeier ML, Leitges M, Kispert A, Camper SA. 2008. WNT signaling affects gene expression in the ventral diencephalon and pituitary gland growth. Dev Dyn 237:1006–1020.

Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrissey EE, Taranova O, Pevny LH, Hogan BL. 2007. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 134:2521–2531.

Sanada Y, Yoshida K, Ohara M, Oeda M, Konishi K, Tsutani Y. 2006. Histopathologic evaluation of stepwise progression of pancreatic carcinoma with immunohistochemical analysis of gastric epithelial transcription factor SOX2: comparison of expression patterns between invasive components and cancerous or nonneoplastic intraductal components. Pancreas 32:164–170.

Schmitz M, Temme A, Sennet V, Ebner R, Schwind S, Stevanovic S, Wehner R, Schackert G, Schackert HK, Fussel M, Bachmann M, Rieber EP, Weigle B. 2007. Identification of SOX2 as a novel glioma-associated antigen and potential target for T cell-based immunotherapy. Br J Cancer 96:1293–1301.

Scheider A, Bardakjian T, Reis LM, Tyler RC, Semina EV. 2009. Novel SOX2 mutations and genotype-phenotype correlation in anophthalmia and microphthalmia. Am J Med Genet 149:2706–2715.

Schoenhals M, Kassambara A, De VJ, Hose D, Moreaux J, Klein B. 2009. Embryonic stem cell markers expression in cancers. Biochem Biophys Res Commun 383:157–162.

Wang P, Liang X, Yi J, Zhang Q. 2008. Novel SOX2 mutation associated with ocular coloboma in a Chinese family. Arch Ophthalmol 126:709–713.

Zorn AM, Barish GD, Williams BO, Lavender P, Klymkowsky MW, Varmus HE. 1999. Regulation of Wnt signaling by Sox proteins: XSox17 alpha/beta and XSox3 physically interact with beta-catenin. Mol Cell 4:487–498.