SHORT COMMUNICATION

Mutation spectrum and phenotypic variation in nine patients with *SOX2* abnormalities

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Multiple mutations in *SOX2* have been identified in patients with ocular anomalies and/or pituitary dysfunction. Here, we identified *SOX2* abnormalities in nine patients. The molecular defects included one missense, one nonsense and four frameshift mutations, and three submicroscopic deletions involving *SOX2*. Three of the six mutations and all deletions were hitherto unreported. The breakpoints determined in one deletion were located within *Alu* repeats and accompanied by an overlap of 11 bp. Three of the six mutations encoded *SOX2* proteins that lacked *in vitro* transactivation activity for the *HESX1* promoter, whereas the remaining three generated proteins with ~15–~20% of transactivation activity. All cases manifested ocular anomalies of various severities, together with several complications including arachnoid cyst and hamartoma. There was no apparent correlation between the residual activity and clinical severity. The results indicate that molecular defects in *SOX2* are highly variable and include *Alu* repeat-mediated genomic rearrangements. Our data provide further evidence for wide phenotypic variation of *SOX2* abnormalities and the lack of genotype–phenotype correlation in patients carrying *SOX2* lesions.

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SOX2 (NP_003097.1) has a critical role in the development of the eye, pituitary and central nervous system through transactivation of multiple genes including *HESX1*.^{1–3} Haploinsufficiency of *SOX2* (NM_003106.3) leads to anophthalmia/microphthalmia and pituitary dysfunction, in addition to various neuronal defects such as mental retardation, brain malformation and hearing loss.^{3–7} Although >80 patients with *SOX2* abnormalities have been reported to date,^{3–11} current understanding of mutation spectrum and phenotypic determinants of this condition remains fragmentary. In fact, *in vitro* functional assays have been performed only for a small number of mutations.^{3,11–13} Furthermore, whereas Kelberman *et al.*¹² found no obvious genotype–phenotype correlation, Schneider *et al.*¹⁴ reported that patients with missense mutations had milder ocular phenotypes than those with nonsense or frameshift mutations.

Here, we identified *SOX2* abnormalities in nine patients (cases 1–9). This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and performed after obtaining written informed consent. Molecular defects in cases 1–9 were identified through sequencing and copy number analyses of *SOX2* for 37 patients with ocular anomalies and

15 patients with pituitary dysfunction and normal eyes (Supplementary Table S1). Mutations in the coding region were examined by direct sequencing, and copy number abnormalities were analyzed by multiplex ligation-dependent amplification and array comparative genomic hybridization. Detailed methods are provided in Supplementary Table S2. Cases 1–6 carried heterozygous intragenic mutations, whereas cases 7–9 had heterozygous submicroscopic deletions involving *SOX2*. Cases 1–9 invariably manifested developmental defects of eyes, in addition to multiple complications including arachnoid cyst and hamartoma (Table 1 and Supplementary Table S3). The ocular phenotypes included unilateral and bilateral microphthalmia, bilateral coloboma and bilateral anophthalmia. The *SOX2* mutations in cases 1–6 consisted of three previously reported mutations (c.70_89del20, c.70_86del17 and c.480C>G) and three novel mutations (c.235T>C, c.244_245delTT and c.402delC) (Figure 1a). None of the six mutations have been registered as polymorphisms in the single-nucleotide polymorphism database (dbSNP, <http://www.ncbi.nlm.nih.gov/>). *In vitro* reporter assays using a vector containing the *HESX1* promoter indicated that c.235T>C, c.402delC and c.480C>G encoded proteins with ~15–

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Table 1 Molecular and clinical findings of cases 1–9

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Age at examination	3 Months	13 Days	1 Year	19 Years	7 Years	1 Year	12 Years	1 Year	19 Years
Sex	Male	Male	Female	Female	Female	Female	Female	Male	Male
<i>Molecular findings</i>									
SOX2 mutation (cDNA)	c.235T>C	c.480C>G	c.70_89del20 ^a	c.70_86del117 ^b	c.244_245delTT	c.402delC	Gene deletion	Gene deletion	Gene deletion
SOX2 mutation (protein)	p.W79R	p.Y160X	p.N24fsX88	p.N24fsX89	p.L82fsX94	p.G135fsX153	Deletion	Deletion	Deletion
Parental origin of the mutation/deletion	NE	NE	<i>De novo</i>	NE	<i>De novo</i>	Father mosaic	NE	NE	<i>De novo</i>
<i>In vitro</i> transactivating activity	Hypomorphic	Hypomorphic	Amorphic	Amorphic	Amorphic	Hypomorphic	Null	Null	Null
<i>Clinical findings</i>									
Ocular abnormality (right)	MO	AO	AO	AO	severe MO	MO	severe MO	AO	Retinal fold
Ocular abnormality (left)	Normal	AO	AO	AO	severe MO	MO	severe MO	AO	Optic disc coloboma
Developmental delay	No	Unknown	No	No	Yes	Yes	Yes	Yes	Yes
Prenatal growth	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	LGA
Postnatal growth failure	Yes	Unknown	Yes	Yes	Yes	Yes	Yes	Yes	Normal
Other complication	Esophageal atresia	None	None	None	Hemihypertrophy	None	Seizure	None	None
Pituitary hormone deficiency	LH, FSH	NE	NE	LH, FSH, GH	NE	NE	LH, FSH, GH	LH, FSH, GH	LH, FSH
Brain MRI findings	Arachnoid cyst	Dandy-Walker syndrome	Hamartoma	Pituitary hypoplasia	Normal	White matter signal abnormality	Ectopic posterior lobe	Pituitary hypoplasia	NE

Abbreviations: AO, anophthalmia; FSH, follicle-stimulating hormone; GH, growth hormone; LGA, large for gestational age; LH, luteinizing hormone; MO, microphthalmia, MRI, magnetic resonance imaging; NE, not examined.

^ade/AACTCCACGGCGGGCGGC.^bde/AACTCCACGGCGGGCGGC.

~20% transactivation activity, whereas the remaining three generated proteins that lacked the activity (Figure 1b). Of the six mutations, c.70_89del20, c.70_86del17 and c.244_245delTT affected the possible nuclear localization signals predicted from mouse data, and therefore seemed to disturb the intracellular localization of the SOX2 protein (Figure 1a).¹⁵ Furthermore, it remained possible that mRNAs of the nonsense and frameshift mutations undergo early degradation *in vivo*. The deletions in cases 7–9 affected ~1.0 to ~2.5 Mb genomic regions at 3q26–27 including SOX2. These deletions overlapped with, but were not identical to, previously reported deletions (Figure 2).^{4,7–9,12–14,16} Sequences at the fusion junction were characterized in case 7, showing that the two breakpoints resided within *Alu* repeats and shared an overlap of 11 bp (Figure 2).

Several matters are noteworthy. First, molecular lesions in cases 1–9 were heterogeneous and included six point mutations and three submicroscopic deletions. These data support a broad mutation spectrum of SOX2 abnormalities.^{8,9} Notably, we identified submicroscopic deletions involving SOX2 in three cases. As multiple microdeletions at 3q26–27 have been reported in patients with ocular anomalies (Figure 2),^{4,7–9,12–14,16} it is possible that the genomic region around SOX2 represents a hotspot for chromosomal rearrangements. In this regard, it is noteworthy that the sequence at the fusion junction in case 7 is consistent with non-allelic homologous recombination that occurs between two homologous sequences or replication-based errors that are usually associated with microhomology at the fusion junction.¹⁷ Furthermore, the two breakpoints of this deletion resided within *Alu* repeats, which facilitate both recombination- and replication-mediated errors.^{18,19} These data imply that *Alu* repeats may have a role in the high frequency of deletions at 3q26–27. Second, cases 1–9 manifested several complications in addition to ocular abnormalities. Importantly, seven cases manifested brain anomalies including arachnoid cyst and hamartoma. In this regard, Alatzoglou *et al.*¹³ recently described pituitary tumors in two patients with SOX2 haploinsufficiency. Thus, SOX2 abnormalities seem to be associated with various types of developmental defects and tumors in the brain. Third, SOX2 lesions were identified in 9 of 37 patients with ocular abnormalities, but were absent from 15 patients with pituitary dysfunction and normal eyes. The results are consistent with the previous reports that SOX2 abnormalities account for 10–20% of the etiology of anophthalmia/microphthalmia and rarely result in pituitary dysfunction without eye abnormalities.^{4,5,8,9,11} These data can be explained by assuming that, during development, the eye is highly sensitive to reduced activity of SOX2. Nevertheless, as gonadotropin deficiency was observed in all of our mutation-positive cases, SOX2 appears to be indispensable for the function of the hypothalamus–pituitary axis. Lastly, no apparent genotype–phenotype correlation was found in our patient cohort. Although ocular phenotypes were relatively mild in cases 1 and 6 with hypomorphic mutations and obviously severe in cases 3 and 4 with amorphic mutations, bilateral anophthalmia was also observed in case 2 with a hypomorphic mutation and mild coloboma with normal visual activity was seen in case 9 with SOX2 deletion. Similarly, the occurrence of pituitary dysfunction, mental retardation and short stature was not associated with the mutation types. The lack of correlation between residual activity and phenotypic severity is consistent with the previously proposed notion that haploinsufficiency of developmental genes is usually associated with a wide range of penetrance and expressivity.²⁰ Collectively, the present study argues for a broad spectrum of SOX2 lesions and indicates for the first time that *Alu* repeat-mediated genomic rearrangements at 3q26–27 account for a part of the etiology

Figure 1 The position and *in vitro* function of *SOX2* mutations identified in the present study. (a) Chromatograms of the mutations. Subcloned wild-type (WT) and mutant (MT) sequences are shown. Deleted nucleotides are shaded in gray and mutated nucleotides are indicated by asterisks. The yellow boxes indicate the position of two putative nuclear localization signals (NLSs) predicted from mouse data.¹⁵ (b) *In vitro* reporter assay using a luciferase vector containing the *HESX1* promoter. The results are expressed as the mean $\pm$ 1 s.d. Asterisks indicate the statistical significance of the results ($P \leq 0.05$). The relative fold activation of c.70_89del20, c.70_86del17 and c.244_245delTT was similar to that of the empty vector (empty). The relative percentages of fold activation of c.235T>C, c.402delC and c.480C>G to that of the WT *SOX2* were 13.8%, 15.4% and 18.4%, respectively.

Figure 2 Submicroscopic deletions identified in the present and previous studies. Upper panel: genomic structure of the 3q26-27 region and the position of previously reported deletions. The position of *SOX2* is indicated by a red box and the deletions are depicted by black bars. Genomic positions of the genes and deletions refer to NCBI database (<http://www.ncbi.nlm.nih.gov/>). Cen, centromere; tel, telomere. Lower panel: comparative genomic hybridization analysis of cases 7–9 from the present study and the sequence at the fusion junction in case 7. The black, red and green dots denote signals indicative of the normal, increased ($> +0.5$) and decreased (< -1.0) copy numbers, respectively. Estimated sizes and positions of the heterozygous deletions are shown.

of SOX2 haploinsufficiency. Our data provide further evidence for the wide phenotypic variation and the lack of genotype–phenotype correlation in patients with SOX2 abnormalities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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