Mutations in *SOX2* cause anophthalmia

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A submicroscopic deletion containing *SOX2* was identified at the 3q breakpoint in a child with t(3;11)(q26.3;p11.2) associated with bilateral anophthalmia. Subsequent *SOX2* mutation analysis identified *de novo* truncating mutations of *SOX2* in 4 of 35 (11%) individuals with anophthalmia. Both eyes were affected in all cases with an identified mutation.

Bilateral anophthalmia is the rarest and most severe form of structural eye malformation. Constitutional chromosome deletions involving 3q27 have been identified in three unrelated individuals with anophthalmia[1,2]. *De novo* apparently balanced reciprocal translocations involving 3q27 were reported in two cases[3,4] of severe bilateral microphthalmia and microphthalmia/anophthalmia.

We obtained a sample of fixed cell suspension from a female infant[4] with isolated bilateral anophthalmia and a *de novo* t(3;11)(q27;p11.2), with the aim of mapping the 3q27 breakpoint using fluorescence *in situ* hybridization (FISH). Because we could identify only a few cells in metaphase in this sample and could not obtain a fresh sample, conventional FISH analysis was not possible. But by co-hybridizing the sample with a 3q paint[5], we were able to unambiguously locate locus-specific BAC probes relative to the breakpoint. BAC signals that co-localized with the two larger domains (normal 3q & derivative 3) mapped centromeric to the breakpoint; those sited in a large and a small 3q domain (normal 3q & derivative 11) were telomeric to the breakpoint; BACs crossing the breakpoint give signals in all three domains (Fig. 1*a*) . Using this strategy, we found that BAC clone RP11-225N10 spanned the breakpoint. Using FISH analysis with BACs flanking the breakpoint, we identified a deletion of roughly 740 kb between RP11-4B14 and RP11-431C22 on the der(3) chromosome (Fig. 1*b*). This deletion was centromeric to the translocation breakpoint and separated from it by roughly 600 kb of non-deleted DNA (Fig. 1*b* and Supplementary Fig. 1 online).

The BAC spanning the translocation breakpoint contained no obvious candidate genes for association with anophthalmia. The available sequence from the deleted region was incomplete, showing only one annotated gene with homology to the 60S ribosomal protein L7a. The gene *SOX2* (encoding the sex determining region Y–box 2) had been previously mapped to 3q26.3–q27 by FISH[6] but could not be identified in the Ensembl assembly. *SOX2* is a transcription factor with site- and stage-specific expression in the developing eye and nervous system[7–10] and with a regulatory role in lens development[9], making it a good candidate gene for association with severe structural eye defects.

BLAST searches using the 3′ region of the sheep ortholog of *SOX2* identified a series of human BAC clones that contained the single-exon gene *SOX2* by both PCR and Southern-blot analysis. We found that four of these BACs (RP11-335I3, RP11-349O18, RP11-203N24 and RP11-223B4) were deleted or partially deleted in the individual with t(3;11). During the course of this study, 217 kb of complete sequence from overlapping BAC clones RP11-43F17 and RP11-379M20 became available. This sequence contained the open reading frame (ORF) of *SOX2*, which codes for 317 amino acids.

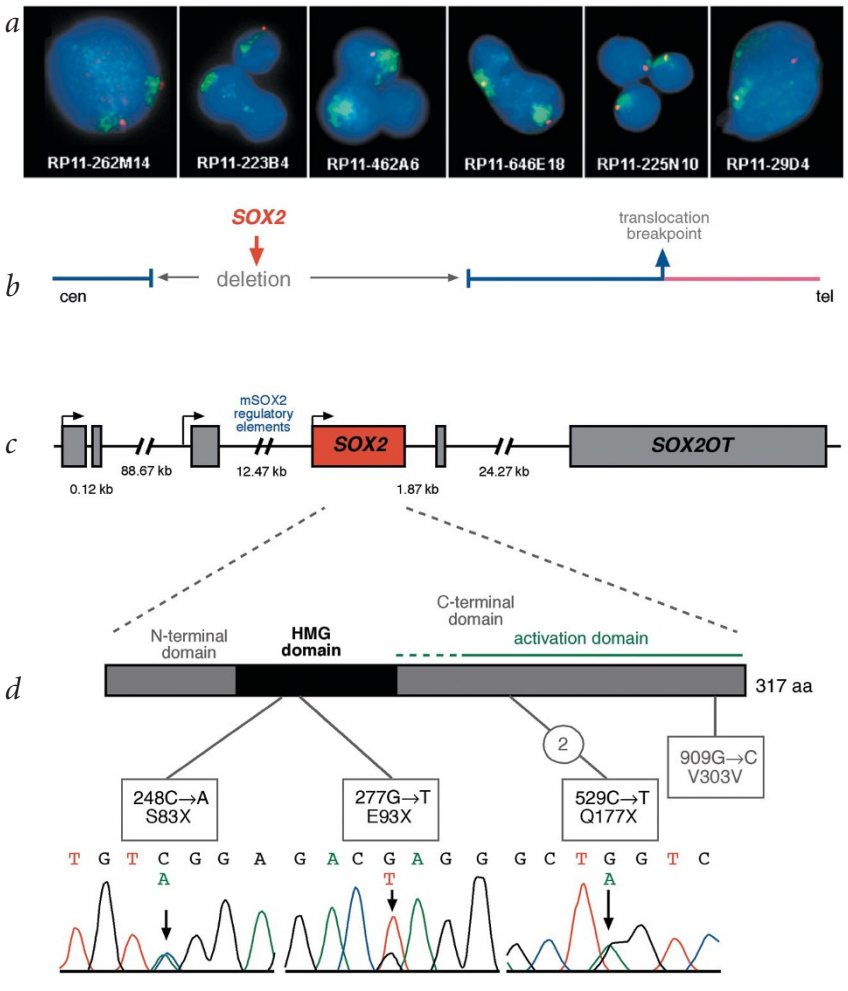

Fig. 1 Mutations in *SOX2* associated with anophthalmia. See Supplementary Note 1 online. ***a***, Interphase nuclei (some multilobed granulocytes) from the individual with t(3;11) and anophthalmia hybridized with a 3q chromosome paint (green signals) and the named BAC clones (red signals). The two large green domains represent the normal chromosome 3 and the der(3) chromosome; the small green domain is the der(11). The left-to-right order of the BAC clones is centromeric to telomeric. RP11-262M14 and RP11-646E18 map centromeric to the breakpoint. RP11-223B4 and RP11-463A6 are deleted. RP11-225N10 crosses the translocation breakpoint. RP11-29D4 maps telomeric to the translocation breakpoint. ***b***, An ideogram of the complex genomic rearrangement surrounding the translocation breakpoint in this individual. ***c***, Cartoon representation of the genomic region surrounding the single-exon *SOX2* gene, which lies within an intron of a conserved, seemingly non-coding gene, *SOX2OT*. ***d***, Electropherograms showing the three *de novo*, heterozygous nonsense mutations that were detected in four unrelated children. The position of the protein truncation with respect to the known functional domains is indicated on the cartoon of the SOX2 protein of 317 amino acids.

Table 1 • Clinical features associated with nonsense mutations in *SOX2*

Mutation	Sex	Right eye	Left eye	Extraocular phenotype
277G→T (E93X)	F	Anophthalmia	Microphthalmia and sclerocornea	Proximal myopathy, normal intelligence
248C→A (S83X)	F	Anophthalmia (small remnant at orbital apex)	Microphthalmia with persistent pupillary membrane	Spastic diplegia, learning difficulties, seizures
529C→T (Q177X)	M	Anophthalmia	Anophthalmia	Hypospadias, hypotonia, delayed motor development, febrile convulsions
529C→T (Q177X)	M	Anophthalmia (small remnant at orbital apex)	Anophthalmia (small remnant at orbital apex)	Microcephaly, cryptorchidism, micropenis, sensorineural deafness, learning difficulties (possibly due to bacterial meningitis); normal MRI

F, female; M, male; MRI, magnetic resonance imaging.

Genomic analysis indicated that *SOX2* lies in an intron of another gene, which we called *SOX2OT* (*SOX2* overlapping transcript (non-coding RNA)). *SOX2OT* has at least five exons and produces a mRNA of roughly 3.4 kb from the same strand as *SOX2* (Fig. 1*c*). It seems to be a non-coding gene with no ORF that encodes a peptide of more than 70 amino acids. *SOX2OT* cDNA is evolutionarily conserved, with 88% nucleotide identity between the human cDNA and available mouse expressed-sequence tags. The sequence over the whole genomic region (roughly 40 kb) encompassing the *SOX2OT* transcription unit, within which *SOX2* is embedded, is highly conserved (see Supplementary Fig. 2 online). Non-coding RNAs often have complex regulatory roles in the cell[11], suggesting that *SOX2OT* may be involved in the transcriptional regulation of *SOX2* in some tissues.

To test whether haploinsufficiency for *SOX2* might have a specific and crucial role in human eye development, we carried out mutation analysis using denaturing high-performance liquid chromatography (DHPLC). We tested 102 individuals with microphthalmia (n = 46), anophthalmia (n = 35) and coloboma (n = 21) without visible cytogenetic abnormalities after the study was approved by the Multicentre Research Ethics Committee for Scotland and London. In total, we detected ten DHPLC shifts in 10 of 102 (9.8%) of these cases. Five of these shifts were due to a heterozygous change (976G→A) in *SOX2* 3′ UTR that we also observed in 5 of 88 (5.7%) unrelated control individuals. The other five unrelated individuals each had a mutation in the *SOX2* coding region.

One mutation involved a neutral change in the third position of codon 303 (V303V) and was inherited from the individual's phenotypically normal mother; this mutation is assumed to be non-pathogenic. We identified two nonsense amino-acid changes (S83X and E93X) in the HMG box (high-mobility group DNA-binding domain) at CpG dinucleotides; these are predicted to ablate DNA binding. We observed a recurrent *de novo* nonsense mutation (529C→T resulting in the amino-acid change Q177X) in two unrelated individuals. Q177X occurs C-terminal to the HMG box but removes 141 amino acids, including much of the highly conserved SOX B1 subgroup activation domain[12]. Each of these nonsense mutations probably results in loss of function (Fig. 1*d*).

The parents of each individual with a mutation in *SOX2* had normal *SOX2* sequence (see Supplementary Fig. 3 online). We confirmed family structure using a panel of 12 autosomal microsatellite markers. No mutations were identified in 176 unrelated control chromosomes from unaffected adults living in Scotland. We excluded large genomic rearrangements around *SOX2* using FISH analysis in 23 cases for which cell lines were available.

Clinically, the individuals with nonsense mutations in *SOX2* presented with anophthalmia (two with bilateral anophthalmia and two with unilateral anopthalmia and contralateral microphthalmia). The associated extraocular abnormalities are variable and include male genital tract anomalies, myopathy and spastic diplegia (Table 1). It is not clear how these phenotypes are related to expression and function of *SOX2*. No missense mutations were detected in individuals with the many milder eye malformations that we screened, suggesting that missense changes may give rise to different phenotypes. Mice heterozygous with respect to a targeted *Sox2*-null mutation show no eye phenotype whereas homozygotes die early in gestation[13]. Such human–mouse differences in phenotypes associated with haploinsufficiency are not uncommon.

Accession numbers. Sheep *SOX2* ortholog, AQ473552; BAC clone RP11-43F17, AC117415; BAC clone RP11-379M20, AC125613; *SOX2OT* (*SOX2* overlapping transcript), AL157425.

Note: Supplementary information is available on the Nature Genetics website.

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Competing interests statement

The authors declare that they have no competing financial interests.

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