Parent-of-origin effects in SOX2 anophthalmia syndrome

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Purpose: Sex determining region Y (SRY)-box 2 (SOX2) anophthalmia syndrome is an autosomal dominant disorder manifesting as severe developmental eye malformations associated with brain, esophageal, genital, and kidney abnormalities. The syndrome is usually caused by de novo mutations or deletions in the transcription factor SOX2. To investigate any potential parental susceptibility factors, we set out to determine the parent of origin of the mutations or deletions, and following this, to determine if birth order or parental age were significant factors, as well as whether mutation susceptibility was related to any sequence variants in cis with the mutant allele.

Methods: We analyzed 23 cases of de novo disease to determine the parental origin of SOX2 mutations and deletions using informative single nucleotide polymorphisms and a molecular haplotyping approach. We examined parental ages for SOX2 mutation and deletion cases, compared these with the general population, and adjusted for birth order.

Results: Although the majority of subjects had mutations or deletions that arose in the paternal germline (5/7 mutation and 5/8 deletion cases), there was no significant paternal bias for new mutations (binomial test, p=0.16) or deletions (binomial test, p=0.22). For both mutation and deletion cases, there was no significant association between any single nucleotide polymorphism allele and the mutant chromosome (p>0.05). Parents of the subjects with mutations were on average older at the birth of the affected child than the general population by 3.8 years (p=0.05) for mothers and 3.3 years (p=0.66) for fathers. Parents of the subjects with deletions were on average younger than the general population by 5.0 years (p=0.22) for mothers and 2.1 years (p=0.19) for fathers. Combining these data, the difference in pattern of parental age between the subjects with deletions and mutations was evident, with a difference of 6.5 years for mothers (p=0.05) and 5.0 years for fathers (p=0.22), with the mothers and fathers of subjects with mutations being older than the mothers and fathers of subjects with deletions. We observed that 14 of the 23 (61%) affected children were the first-born child to their mother, with 10/15 of the mutation cases (66%) and 4/8 deletion cases (50%) being first born. This is in comparison to 35% of births with isolated congenital anomalies overall who are first born (p=0.008).

Conclusions: Sporadic SOX2 mutations and deletions arose in both the male and female germlines. In keeping with several genetic disorders, we found that SOX2 mutations were associated with older parental age and the difference was statistically significant for mothers (p=0.05), whereas, although not statistically significant, SOX2 deletion cases had younger parents. With the current sample size, there was no evidence that sequence variants in cis surrounding SOX2 confer susceptibility to either mutations or deletions.

Developmental eye malformations, including anophthalmia (clinical absence of the eye) and microphthalmia (small eye), are a major cause of visual impairment worldwide. These conditions are clinically heterogeneous, and can manifest as either purely ocular defects, or for more than half of cases, in association with systemic anomalies [1]. Frequently, the cases display non-Mendelian inheritance patterns, reflecting the likely importance of genetic background and environmental influences. The first causative genes, most transcription factors that control eye morphogenetic pathways, are beginning to be identified, with dominant, recessive, X-linked, and oligogenic mechanisms represented [2–14].

Evidence from knockout gene experiments in mice (JAX), cytogenetic events associated with eye anomalies, and the number of human syndromes that include anophthalmia or microphthalmia as a clinical feature (Oxford Dysmorphology Database 2.1), suggest that at least 200 human anophthalmia-microphthalmia (AM) syndromes may eventually be defined. Correlations between AM and parental exposure to environmental factors around the time of conception or early pregnancy have been postulated [15]. However, epidemiological studies, by their nature, group all AM conditions together regardless of cause (see for example [16–19]), whereas each might represent a distinct genetic pathway with its own set of parameters and risk factors. Identification of the molecular basis of individual syndromes provides an opportunity to explore how different mutational events arise. The first step is to determine parental origin as this is a prerequisite to understanding periconception or gestational risk factors that contribute to disease.
Herein we have set out to determine the parent of origin for one of the earliest anophthalmic disorders to be genetically defined, sex determining region Y (SRY)-box 2 (SOX2; OMIM 184429) anophthalmia syndrome. This condition is characterized by severe, often bilateral, ocular malformations including anophthalmia or microphthalmia with coloboma or cyst, optic nerve hypoplasia, and rarely, retinal dystrophy [5,6,10,20]. Clinical features also include several extraocular features, including poor growth, cognitive deficit, motor disorder, seizures, sensorineural hearing loss, mesial temporal brain malformations, anterior pituitary hypoplasia, horseshoe kidney, and male (and possibly female) genital abnormalities [5,6,10,20–22]. The syndrome is usually caused by haploinsufficiency of SOX2, a transcription factor with a key regulatory role in lens development [23,24]. Four families have been described where SOX2 mutations were inherited from a gonosomal mosaic mother [25–28]. All remaining reported cases of SOX2 mutations and deletions have occurred de novo. However, there is no information about the parental origin of the mutation or deletion for any of these cases. We used haplotype analysis to determine the parent of origin of SOX2 mutation and deletion cases, and to investigate their relationship with parental age and birth order. We also sought to determine whether any sequence variants adjacent to the SOX2 gene were associated with a susceptibility to mutation or deletion.

**METHODS**

**Cases:** Informed consent for genetic and phenotypic analysis was obtained from the patient and parents, in accordance with approval by the Cambridgeshire I Research Ethics Committee 04/Q0104/129. Paternity was confirmed using the PowerPlex® 16 System (Promega, Southampton, UK). Cases 1–8 with SOX2 deletions and cases 9–19 with SOX2 mutations have been previously described [5,6,10]. The location of mutations and deletions are shown in Table 1. SOX2 deletion and mutation cases were identified as de novo following analysis of peripheral blood samples; however, it is not possible to exclude parental gonosomal mosaicism. Case 19 has bilateral anophthalmia with microopenis, agenesis of the corpus callosum, and severe developmental delay, and is most likely to be the same case as previously reported [22]. The proband has a de novo heterozygous c.479delA (p.Tyr160Serfs*4) mutation. Cases 20–23 are previously unreported de novo SOX2 mutations, as determined by analysis of peripheral blood. Case 20 is a male with right microanterior segment, sclerocornea, thin lens, posterior staphyloma with hand movement vision, and left extreme microphthalmia (axial length 10 mm) with congenital aphakia, short stature (normal pituitary function), low weight, unusual gait, muscle weakness, small fifth finger, dysmorphic features, slightly prominent nose, and has experienced fainting fits. He had a c.582_592dup (p.His198Argfs*9) mutation. Case 21 is a boy with isolated bilateral anophthalmia. There were no other systemic features apart from a slightly high arched palate. The proband has a c.143_144delinsAA mutation (p.Phe48*). Case 22 is a girl with isolated bilateral anophthalmia. She has a de novo c.70_89del (p.Asn24Argfs*65) mutation. Case 23 is a boy born at term with a normal birthweight, bilateral severe microphthalmia associated with funnel retinal detachments, and delayed motor development. He has a de novo c.542delC mutation (p.Pro181Argfs*22).

**Determining parent of origin:**

**Single nucleotide polymorphism genotyping**—Single nucleotide polymorphisms (SNPs) surrounding the SOX2 genomic loci were genotyped to determine the parental origin of sporadic SOX2 mutations and partial or whole gene deletions. To trace the parental origin of each SOX2 deletion, we identified cases where affected subjects failed to inherit an SNP allele from one parent. We genotyped 24 SNPs within 50 kb of the SOX2 gene in subjects 1–8 and their parents. A molecular haplotyping approach (described below) was used to determine the parent of origin for the SOX2 mutations.

Genomic DNA was prepared from peripheral blood samples using a QIAamp DNA Blood Mini kit (Qiagen, Crawley, UK). For mutation and deletion cases, we genotyped the following SNPs: rs13070015, rs9290727, rs4434184, rs6806029, rs12496378, rs12487748, rs12497248, rs35788479, rs35095647, rs11915160, rs4575941, and rs4459940 (SNP). For deletion cases, we also genotyped SNPs: rs13074951, rs13097472, rs1558797, rs1558798, rs34961466, rs36062376, rs4855037, rs6443761, rs6443762, rs6765739, rs7610679, and rs7633815. PCR products were generated for SNPs using the primers detailed in Table 2. PCR amplification was performed in a 50 µl reaction volume containing 100 ng DNA, 0.1 µM each primer, 5 µl 10× reaction buffer, 2 µl 25 mM MgCl2, 0.2 mM dNTPs, and 2.5 Units HotStarTaq DNA polymerase (Qiagen). Cycling parameters were 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were treated with ExoSapIT (USB) and sequenced using standard techniques.

**Molecular haplotyping**—We used a molecular haplotyping approach [29] to determine the parental origin of each SOX2 mutation. We genotyped 12 SNPs within 20 kb of the SOX2 gene in the SOX2 mutation subjects and their parents, and identified at least one informative SNP for seven subjects. For each of the seven informative subjects, genomic DNA at limiting dilution was used to generate a panel of 45 aliquots (and one negative control). Each aliquot was amplified using primers for both the SOX2 CoDing Sequence (CDS) and an informative SNP locus (rs11915160, rs4459940, or rs4575941) in a single multiplex PCR. PCR products were then diluted and the SOX2 CDS and SNP locus were amplified in separate monoplex PCR reactions using heminested primers. For DNA samples, 47/94±6.19 (standard deviation [SD]) aliquots were positive
for the SOX2 CDS or the informative SNP locus. Assuming a Poisson distribution of DNA fragments among the samples [29], this implies a mean of 0.71±0.14 (SD) autosomal genomes per aliquot. Aliquots positive for the presence of both the SOX2 CDS and the informative SNP locus were then sequenced to determine which SNP variant was associated with the mutant SOX2 allele. A small number of aliquots were derived from both haplotypes because they were heterozygous for either the SOX2 mutation or the SNP locus; these did not contribute to determine parental origin. Individual haplotypes were reconstructed by inspection of the data and the parental origin determined in each case. For each subject, the haplotype was supported by odds of at least 10⁹:1.

The multiplex first-phase PCR contained primers for SOX2 and one informative SNP (rs11915160, rs4459940, or rs4575941). Alternative SOX2 primers were used depending on the position of the mutation. The first-phase PCR was performed in a 10 µl reaction containing 1 µl DNA (at one genome equivalent [3 pg/µl]), 0.04 µM each external-forward and -reverse primer, 10X reaction buffer, 1 mM MgCl₂, 0.2 mM dNTPs and 0.5 Units HotStarTaq DNA polymerase (Qiagen). Cycling conditions were 94 °C for 15 min followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. First-phase reaction products were diluted to 150 µl with water and 3 µl aliquots were used as template in second-phase PCR reactions to independently assay SOX2 and each SNP. Second-phase PCR was performed in a 20 µl reaction containing template DNA, 0.2 µM each internal-forward and external-reverse primer, 2 µl 10X reaction buffer, 1 mM MgCl₂, 0.2 mM dNTPs, and 1 Unit HotStarTaq DNA polymerase (Qiagen). Cycling conditions were the same as for the first-phase PCR. Products were analyzed by gel electrophoresis for the presence/absence of the expected PCR product. PCR products from aliquots positive for SOX2 and the SNP locus were treated with ExoSapIT (USB, High Wycombe, UK) and sequenced using standard techniques [11,12].

The confidence that can be assigned to the linkage phase between the SOX2 and SNP loci was estimated using calculations described by Konfortov et al. [29]. This measure depends on the physical distance between two loci, the average fragment size of DNA in the aliquots, the number of autosomal genomes per aliquot, and the number of genotyping results that agree or conflict with a given haplotype phase. The physical distance between each SOX2 mutation and the SNP loci was calculated for each assay using genomic positions from the UCSC genome browser [30]. Template DNA was prepared using the QIAamp DNA Blood Mini kit (Qiagen), which predominantly yields fragments of 20–30 kb; therefore, we conservatively estimated average DNA fragment size at 20 kb. The number of autosomal genomes per aliquot was estimated for each sample from the total number of positive

| Case | SOX2 gene change | Reference |
|------|-----------------|-----------|
| 1    | Partial gene deletion | [10] |
| 2    | Whole gene deletion | [10] |
| 3    | Whole gene deletion | [10] |
| 4    | Whole gene deletion | [10] |
| 5    | Whole gene deletion | [10] |
| 6    | Whole gene deletion | [10] |
| 7    | Whole gene deletion | [10] |
| 8    | Whole gene deletion | [10] |
| 9    | c.529C>T; p.Q177X | [5,6] |
| 10   | c.53 C>A mutation, p.S18X | [10] |
| 11   | c.248C>A, p.S83X | [5,6] |
| 12   | c.70del20, p.N24fs88X | [10] |
| 13   | c.480C>G, p.Y160X | [10] |
| 14   | c.285dupG, p.K95fs109X | [10] |
| 15   | c.529C>T; p.Q177X | [5,6] |
| 16   | c.290T>C; p.L97P | [6] |
| 17   | c.70del20 p.N24fs88X | [10] |
| 18   | c.188delA p.N63fs101X | [10] |
| 19   | c.479delA, p.S159fs163X | This study; same case likely published [22] |
| 20   | c.582–592dup, (p.His198Argfs*9) | This study |
| 21   | c.143–144TC>AA, (p.Phe48*) | This study |
| 22   | c.70del20 p.N24fs88X | This study |
| 23   | c.542delC, (p.Pro181Argfs*22) | This study |
алиquotas в втором этапе ПЦР используя посапуновскую распределение [29].

**Sequence variant analysis:** To examine whether particular sequence variants predispose individuals to the occurrence of mutations or deletions, we used parental data to determine the genotype of SNPs located on mutant (mutated/deleted) and wild-type (nonmutated/nondeleted) chromosomes. For deletion cases, we analyzed 24 SNPs, and for mutation cases 12 SNPs, previously genotyped for parental origin. We compared the frequency of alleles on mutant versus wild-type chromosomes using Fisher’s exact test.

**Parental age:** To examine parental age effects in SOX2 anophthalmia syndrome, the median ages of parents of affected subjects were compared with median parental age at birth of the general population using the national birth cohorts of England and Wales for the relevant years of birth of the affected children. Data on birth order of the affected children were compared with data from the Congenital Anomalies Register for Oxfordshire, Berkshire and Buckinghamshire (CAROBB), as cases from the England and Wales National Congenital Anomalies System are known to be underascertained and not published by birth order.

**RESULTS**

**Parent of origin:** Deletion cases: The majority of subjects (5/8) had deletions that arose in the paternal germline, but this did not represent a statistically significant paternal bias to the a priori assumption that deletions occur with the same probability on maternal and paternal chromosomes (binomial test, p=0.22).

**Mutation cases:** In the majority of subjects (5/7), the mutation was paternal in origin, although this does not represent a statistically significant paternal bias to the a priori assumption that mutations occur with the same probability on maternal and paternal chromosomes (binomial test, p=0.16).

For both mutation and deletion cases, there was no significant association between any SNP allele and the mutant chromosome (p>0.05). With the current sample size, there is no evidence that in cis sequence variants surrounding SOX2 confer susceptibility to either mutations or deletions.

### Table 2. PCR primers for SNP analysis.

| SNP          | Forward 5′-3′                      | Reverse 5′-3′                          |
|--------------|------------------------------------|----------------------------------------|
| rs13070015   | CCAGAGTTGAGACCACCTTACTG            | GGTTGAGAGAGAGAATAA                     |
| rs9290727    | CAGGCTTTGAATGGCCCTTACG             | GATTTGGAATCGAGAAGAACGAAATTG            |
| rs4434184    | ATCACTCAGAGCGAGCAGATAAG            | GGATTTGCAGAAGCAGAAGATCG               |
| rs6806029/8  | TGCAAAACACATCAGCGCCCTTCTG          | GAGGTAAGGAGAGAGTGCTGTAAGAGG           |
| rs12487748/8 | AGCTGTAGTGAGGATAGATAAATCCTACCCAT  | GACAGGTTCTTGACTGACAGAAG               |
| rs3578847    | TGACGCTTTTGAGTTCGTTCTCACAGAGAAG   | GCCCACTCATTCTGCACTCGAC                 |
| rs35095647   | GTACGCTTTTGAGTTCGTTCTCACAGAGAAG   | CAGCAGGTTCTTGACTGACAGAAG              |
| rs11915160   | GGCTCAAAAGAGCAATGGCTTCCTCAAA      | CTCCTCTCTACCCCTGACAGAAG               |
| rs4575941/4  | CCAGGTTGAGAGAGAGAATAA             | GTTGGAGGAGAGAGAGAGAGAGAGAATAA         |
| rs34961466/8 | CCAAGCCTCTGCAGCCCAAT              | GGTGGAGGAGAGAGAGAGAGAGAGAATAA         |
| rs4855037/8  | GCTTGAAGAGACAGAAGATCG             | GGTGGAGGAGAGAGAGAGAGAGAGAATAA         |
| rs7633815/8  | GAGGTTTCTACCTCTCTCTGTC            | GGTGGAGGAGAGAGAGAGAGAGAATAA           |
| rs6443761/6  | GCGCTACCTCTGCTCCTATGTCCT          | GGTGGAGGAGAGAGAGAGAGAGAATAA           |
| rs6765739    | GAGGCTGTGCTTGGCTAGAGAAG           | GGTGGAGGAGAGAGAGAGAGAGAATAA           |
| rs7610679    | TACAGGAAGACAGAAGATGCTCAAGAAGAG    | GGTGGAGGAGAGAGAGAGAGAGAATAA           |
| SOX2 5′ outer| TACAGGTAATGAGAAGAGAGAAGATCGA     | GGTGGAGGAGAGAGAGAGAGAGAATAA           |
| SOX2 5′ inner| GTAGGAGGAGAGAGAAGATGCTGA          | GGTGGAGGAGAGAGAGAGAGAATAA             |
| SOX2 3′ outer| GGCCTGGAAGAGAGAGAGAAGATCGA       | GGTGGAGGAGAGAGAGAGAGAATAA             |
| SOX2 3′ inner| GACCGTTCCCGGTGTTTCTCT            | GGTGGAGGAGAGAGAGAGAGAATAA             |
| rs11915160 outer | CCAATCCACATCCACTACAG              | GGTGGAGGAGAGAGAGAGAGAATAA             |
| rs11915160 inner | GTACGCTTTTGAGTTCGTTCTCACAGAGAAG | GGTGGAGGAGAGAGAGAGAGAATAA             |
| rs4575941/4  outer | CCAATCCATCCACTACACTACAG          | GGTGGAGGAGAGAGAGAGAGAATAA             |
| rs4575941/4  inner | GTACGCTTTTGAGTTCGTTCTCACAGAGAAG | GGTGGAGGAGAGAGAGAGAATAA               |
| rs3578847    | CCAATCCATCCACTACACTACAG          | GGTGGAGGAGAGAGAGAGAATAA               |
| rs11915160 outer | CCAATCCATCCACTACACTACAG          | GGTGGAGGAGAGAGAGAGAATAA               |
| rs11915160 inner | GTACGCTTTTGAGTTCGTTCTCACAGAGAAG | GGTGGAGGAGAGAGAGAGAATAA               |
| rs4575941/4  outer | CCAATCCATCCACTACACTACAG          | GGTGGAGGAGAGAGAGAGAATAA               |
| rs4575941/4  inner | GTACGCTTTTGAGTTCGTTCTCACAGAGAAG | GGTGGAGGAGAGAGAGAGAATAA               |

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TABLE 3. PARENTAL AGE AT THE BIRTH OF THE ANOPHTHALMIA SUBJECTS COMPARED WITH PARENTAL AGE IN THE GENERAL POPULATION OF BIRTHS FOR ENGLAND AND WALES.

| Parental age comparisons | Number of anophthalmia subjects | Parents of anophthalmia subjects | Parental age at birth for the general population$^3$ | Difference in age (years) | p value$^4$ |
|--------------------------|----------------------------------|-----------------------------------|------------------------------------------------------|---------------------------|------------|
| **Deletions** | | | | | |
| Median (range) paternal age in years | 8 | 30.0 (24.0–34.0) | 32.1 (30.0–32.2) | −2.1$^1$ | 0.19 |
| Median (range) maternal age in years | 8 | 24.5 (21.0–30.0) | 27.5 (26.5–31.0) | −3.0$^1$ | 0.17 |
| **Mutations** | | | | | |
| Median (range) paternal age in years | 13 | 35.0 (18.0–44.0) | 31.7 (29.7–32.4) | +3.3$^1$ | 0.66 |
| Median (range) maternal age in years | 15 | 31.0 (18.0–41.0) | 27.2 (25.1–32.4) | +3.8$^1$ | 0.05 |
| **Paternal age** | | | | | |
| Median (range) paternal age in years of subjects with deletions | 8 | 30.0 (24.0–34.0) | - | +5.0$^2$ | 0.22 |
| Median (range) paternal age in years of subjects with mutation | 13 | 35.0 (18.0–44.0) | - | | |
| **Maternal age** | | | | | |
| Median (range) maternal age in years of subjects with deletions | 8 | 24.5 (21.0–30.0) | - | +6.5$^2$ | 0.05 |
| Median (range) maternal age in years of subjects with mutation | 15 | 31.0 (18.0–41.0) | - | | |

$^1$The difference is the median parental age of anophthalmia subjects minus the median parental age in the general population. $^2$The difference is the median parental age of anophthalmia mutation cases minus the median parental age of anophthalmia deletion cases. $^3$Median of the birth year-specific general population means. $^4$P-values derived from the Mann–Whitney U test.
We were unable to determine parental origin in the remaining eight cases because SNPs were uninformative (4 cases) or DNA samples were unavailable (4 cases).

**Parental age:** Parents of the subjects with mutations were on average older at the birth of the affected child than the general population by 3.8 years (p=0.05) for mothers and 3.3 years (p=0.66) for fathers. In contrast, the parents of the subjects with deletions were on average younger than the general population by 3.0 years (p=0.17) for mothers and 2.1 years (p=0.19) for fathers. The difference in pattern of parental age was evident in the comparison of parental age between the subjects with deletions and mutations, with a difference of 6.5 years for mothers (p=0.05) and 5.0 years for fathers (p=0.22), with the mothers and fathers of subjects with mutations being older than the mothers and fathers of subjects with deletions. We observed that 14 of the 23 (61%) affected children were the first-born child to their mother, with 10/15 of the mutation cases (66%) and 4/8 deletion cases (50%) being first born. This is in comparison to 35% of births with isolated congenital anomalies overall who are first born (p=0.008; P Boyd, CAROBB, personal communication).

**DISCUSSION.**

The parental origin of de novo genetic anomalies depends on several factors. First, the type of genetic defect is often parent specific. Spontaneous base substitutions are thought to occur preferentially in the paternal, rather than the maternal, germline because of the higher number of cell divisions in spermatogenesis than oogenesis [31]. This is supported by evidence from several disorders, where spontaneous base substitutions arise predominantly in the paternal germline [32,33]. In contrast, certain spontaneous chromosomal abnormalities are more commonly maternal in origin, such as those causing Duchenne muscular dystrophy or neurofibromatosis [34,35]. Second, a genetic anomaly might have a selective advantage or disadvantage in male or female germ cells. For example, positive selection of sperm with mutations in FGFR2 in the male germline is thought to contribute to the paternal age effect in Apert syndrome [36]. Third, parental origin might depend on which parent has been exposed to environmental hazards that cause germline haploinsufficiency. The majority of cases are de novo, with more than one individual: c.529C>T (p.Gln177*) and c.70_89del (p.Asn24Argfs*65) in published cases 12 and 17, and the new case 22. The c.70_89del (p.Asn24Argfs*65) mutation has also been noted in eight other published anophthalmia cases summarized by Schneider and colleagues [28], including one pair of monozygotic twins discordant for anophthalmia [26]. The c.70_89del (p.Asn24Argfs*65) mutation results in the deletion of one of a pair of direct 5′-[GCG] 3′ repeats, and the intervening nine nucleotides. The c.70_89del (p.Asn24Glyfs*66) mutation has also been noted in two siblings discordant for anophthalmia [26]. The c.70_89del (p.Asn24Argfs*65) mutation results in the deletion of one of a pair of direct 5′-[GCG] 3′ repeats, and the intervening nine nucleotides. The c.70_89del (p.Asn24Glyfs*66) mutation results in the deletion of one of the 5′-[GCG] 3′ repeats, and the intervening nine nucleotides. We were able to determine parental origin for one of the c.70_89del (p.Asn24Argfs*65) cases in our cohort, and found that the mutation was paternally derived. Previous studies have found described cases of inherited SOX2 mutations or deletions, most likely because of reduced genetic fitness, in addition to male and possibly female genital abnormalities, some related to hypothalamic-pituitary axis dysfunction.
that this type of mutation is predominantly paternal in origin and arises during replication by a mispairing of direct repeats (see [52]).

Sporadic cases of several disorders are associated with older paternal age (reviewed in [32]) or rarely with younger maternal age [53–55]. We found the mutation cases were associated with older parental age, although this was only statistically significant for the mothers. The small number of mutation cases of paternal origin (n=13) means that chance cannot be excluded as an explanation for this finding, but the absence of a paternal age effect is consistent with the absence of a paternal origin bias. One possible explanation for the maternal age association is that DNA repair mechanisms are maternally derived from mRNA and protein in the oocyte. For example, female mice deficient in double-stranded break repair do not efficiently repair double-strand breaks that originally arose in sperm [56,57]. Therefore, the ability of the mother to correct a genetic defect at oogenesis or during the first cell division after zygosis is critical [58], and this may be impaired with increasing age.

In contrast, we found the deletion cases had parents who were on average younger by two to three years than parents in general, and this difference is substantial in population terms. These results were, however, not statistically significant; that is, with only eight cases in total, these comparisons lacked the statistical power to unmask what might be a real and important finding that merits further investigation. Younger maternal age has previously been noted in several disorders, including septo-optic dysplasia [53], cleft lip, central nervous system anomalies, gastroschisis, and skin, upper limb and female genital anomalies [54,59,60]. One possible reason for this is a greater likelihood of the survival of malformed conceptuses in younger women, which is consistent with the finding that the frequency of chromosomally normal spontaneous abortions increases with maternal age [61]. Several studies have also drawn attention to the role of lifestyle factors in the etiology of anomalies associated with younger maternal age. For example, drug and alcohol consumption and cigarette smoking have been implicated in the risk of specific congenital anomalies that tend to occur predominantly in younger women, for example, gastroschisis [60,62] and optic nerve hypoplasia [55,63,64]. With our relatively small number of cases, we were unable to explore further the relationship between lifestyle and/or environmental factors and the occurrence of SOX2 deletions or mutations. Such investigations will require an international collaborative effort to identify sufficient cases to explore this further. However, this would clearly be worthwhile if this is able to shed light on the causal mechanisms leading to SOX2 mutational events, which have such a profound impact on the children and families affected.

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