Screening of the 1 Mb SOX9 5′ control region by array CGH identifies a large deletion in a case of campomelic dysplasia with XY sex reversal

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Campomelic dysplasia (CD; OMIM #114290) is a semi-lethal, autosomal dominant osteochondrodysplasia, characterised by congenital shortening and bowing of the long bones (campomelia) in combination with other skeletal anomalies such as hypoplasia of the scapular and pelvic bones, lack of mineralisation of thoracic pedicles, a missing pair of ribs, and clubbed feet. Severe respiratory distress resulting from Robin sequence, hypoplastic lungs with narrow airways, and a bell shaped narrow thorax are the major cause of death, which mostly occurs during the neonatal period. About two thirds of karyotypic male CD patients show XY sex reversal. As is the case for any of the symptoms, campomelia is not an obligatory feature and is absent in about 10% of the cases, referred to as the acampomelic form of CD (ACD).1,2

Most CD cases have heterozygous de novo mutations in the coding region of the transcription factor gene SOX9 on 17q.1-4 The mutations cause loss of DNA binding or of the transactivation function of SOX9, implying that CD results from haploinsufficiency for SOX9. In accordance with the human phenotype caused by SOX9 mutations, studies in the mouse have shown that Sox9 functions as an essential developmental regulator at various steps of chondrogenesis5-7 and during the initial phase of testis determination and differentiation.6,8,9 Furthermore, heterozygous Sox9 knockout mice recapitulate essentially all the symptoms seen in CD patients but for the sex reversal.5,10

Some CD cases have two intact copies of the SOX9 structural gene but a chromosomal rearrangement in the vicinity of one SOX9 allele. These rearrangements, which include translocations and inversions, occur de novo, and their breakpoints are always 5′ to SOX9. In 11 of these cases, the breakpoints have been mapped from 50 kb up to 950 kb upstream of SOX9.4,9-11 Detailed analysis of the genomic sequence of this 1 Mb region showed it to be devoid of any protein coding gene, suggesting that the chromosomal rearrangements remove one or more cis regulatory elements from an extended SOX9 control region.7,12 In support of this, mice transgenic for human SOX9 spanning YACs showed expression patterns (except in gonads) for the transgene similar to endogenous Sox9 only when the YAC transgene contained a 350 kb sequence upstream of SOX9, but not with a truncated YAC containing only 75 kb of SOX9 5′ flanking sequence.7 By phylogenetic footprinting comparing the genomic sequences of the SOX9 regions of human, mouse, and puffer fish (Takifugu rubripes), we recently identified five conserved sequence elements up to 290 kb 5′ of human SOX9 and showed that 8 of 10 CD translocation breakpoints separate part or all of these elements from SOX9.13

Our mutation detection rate, performed by sequencing of the SOX9 open reading frame (ORF) and of the four exon-intron junctions, is about 90–95% in classical CD/ACD non-translocation/inversion cases. The remaining cases could have a mutation within the proximal promoter, the 5′ or 3′ UTR, or the introns, regions not routinely analysed. They could also have a submicroscopic deletion within the 1 Mb 5′ control region of SOX9. We reasoned that comparative genomic hybridisation (CGH) on a DNA microarray, using large insert clones covering a 2.5 Mb region around SOX9, might be an effective and simple procedure to search for a deletion in such an extended genomic region.

CASES AND METHODS

Case reports

Patient 1 (Neuburg) was the first child of unrelated, healthy parents. At birth, maternal age was 22 years, and paternal age was 23 years. There was no family history of CD. At birth, the patient had severe thoracic hypoplasia, narrow thorax, clubbed feet, and severe respiratory distress. The parents were referred to the genetics department. A complete karyotype showed a normal male karyotype, 46 XY. A study of the SOX9 gene revealed a splice-site mutation (c.692-1G>C) in exon 7 with no parental segregation. DNA microarray analysis by comparative genomic hybridisation (CGH) identified a large deletion in the vicinity of SOX9.
age 26 years. The mother previously had a termination of pregnancy with another partner. Birth weight was 2720 g, length was 49 cm, and occipitofrontal circumference was 37 cm. Apgar scores were 9/3/6. The child had to be intubated directly after birth, and presented with radiological signs typical of CD such as hypoplastic scapulae, narrow bell shaped thorax, 11 pairs of ribs, and hypoplastic scapula. (B) Chest at 7.5 months of age, showing severe scoliosis. (C) Pelvis and lower extremities 1 day after birth, showing vertically narrow iliac bones, hypoplastic ischial and pubic bones, bent femur and slightly bowed tibia and fibula. (D–F) Patient 2. (D) Chest at 13 months of age. Note only mild hypoplasia of the scapula. (E) Lateral cervical spine at 5 years of age, showing kyphosis and partial non-mineralisation of pedicle C2. (F) Pelvis and lower extremities at 5 months of age, showing hypoplastic parts of the ischial and pubic bones, no narrow iliac wings, and no overt bending of the long bones.
he had developed a severe scoliosis (fig 1B), and had to be tube fed continuously. He had severe respiratory problems, which required repeated hospitalisation and from which he died at 10 months of age. His final weight was 4790 g, length 59.5 cm, head circumference 44.7 cm.

Patient 2 (Tours) was born at 39 weeks gestation to healthy, unrelated parents, a 21 year old G1P1 mother and a 22 year old father. Pregnancy was uneventful. Birthweight was 3540 g, and occipitofrontal circumference was 37.5 cm. The child presented at birth with macrocephaly, epicanthic folds, flat nasal bridge, shallow orbits, antverted nostrils, Robin sequence, bilateral clubfoot, and tracheobronchomalacia that initially required tracheotomy. Radiological findings included a mild, non-characteristic hypoplasia of the scapula (fig 1D), and an unusual kyphosis of the cervical spine with partial non-mineralisation of the pedicle C2 (fig 1E). The pelvis and lower extremities showed hypoplastic ischial (ramus) and pubic (inferior ramus) bones, but no narrow iliac wings and no overt bending of the long bones (fig 1F). Ischial and pubic rami were still insufficiently mineralised at 5 years of age. Metacarpophalangeal pattern profile analysis of hand radiographs demonstrated some shortness of metacarpal I (~2.4 SD), moderately short middle phalanges II (~5.6 SD) and V (~2.6 SD), and mild shortness of distal phalanges I, IV, and V, a pattern similar to that described for other CD cases. At 5 years of age, her height was 106 cm (~1 SD), weight 16.5 kg (~0.6 SD), and head circumference 51.5 cm (~1 SD). Retrogadthia had improved and hearing was normal. She has normal female external genitalia and a 46,XY karyotype. At the last follow up, at 5 years and 8 months of age, she was found to be a hyperactive, cheerful girl, attending a regular kindergarten where she showed good social integration. She was still having some problems with pronunciation, but was able to make herself understood.

**Somatic cell hybridisation and FISH analysis**

Mouse–human somatic cell hybrids were established from fusion of a lymphoblastoid cell line from patient 1 with mouse RAG cells (Hprt-), and of skin fibroblasts from patient 2 with mouse RAG (Hprt-) and B82 cells (TK-), as described previously. FISH analysis was performed as previously described.

**PCR typing of microsatellite and STS markers**

Microsatellite markers amplified by PCR with unlabelled primers were analysed on 6% denaturing polyacrylamide gels followed by silver staining. Microsatellite markers amplified by PCR with dye labelled primers were analysed on an ABI Prism 310 automated DNA sequencer using Genotyper software (version 3.7) (both Applied Biosystems). Paternity was tested using the ProfilerPlus kit (Applied Biosystems).

Marker content of somatic cell hybrids was determined using primers for microsatellite and previously published STS markers. For fine mapping of the deletion breakpoint for patient 2, new primer pairs were developed from the genomic sequence and are available upon request (RP markers). Primers used for deletion spanning PCR were RPI7F-Long (5'-ACCCTCGAGGACAATCGATCCAG-3'), and RPI4R-Long (5'-CTTGGACACTGGCCCTAGTGCTG-3'). Standard PCR was performed as previously described. Long range PCR was conducted with the Expand Long Template PCR system (Roche).

**Sequence analysis**

The Vectorette protocol was used to obtain the human insert end sequence of YAC 946E12. The PCR product from the deletion junction of patient 2 was cloned into pCRII-TOPO (Invitrogen) prior to sequencing. Sequence reactions were performed with the Thermosequenase II Dye Terminator Cycle Sequencing kit (Amersham Pharmacia) and analysed on an ABI Prism 310. Conserved sequence elements were identified by BLAST searches of human sequences against the Takifugu sequence release 3 on the (Takife)Gu website (http://tfgu.hgmp.mrc.ac.uk), and against mouse sequences at the NCBI BLAST site (http://www.ncbi.nlm.nih.gov/BLAST). PipMaker analyses were performed at http://bio.cse.psu.edu/pipmaker.

**Microarray printing, hybridisation and data analysis**

BACs and PACs from chromosome 17 and reference BACs from the X chromosome were grown in selective media in an overnight culture and DNA was extracted with the Qiagen Plasmid Midi kit using the manufacturer's protocol for very low copy plasmids. DNA was eluted from the columns with distilled water and spotted onto Corning CMG-GAPSTM coated slides according to the manufacturer’s recommendations. The dot spacing was 500 μm. Each clone was spotted twice in one array and arrays printed in duplicate. Washing and coupling steps were also performed as per the manufacturer’s (Corning) instructions. After a denaturation step in boiling water, the slides were rinsed in 100% ethanol and dried by centrifugation.

The labelling and hybridisation steps were performed according to the protocol of Pollack et al (http://cng.stanford.edu/brown/protocols/4_genomic.html). In brief, 2 μg test and 2 μg reference DNA were labelled with Cy3 and Cy5 dyes (Amersham Pharmacia) using the BioPrime DNA labelling kit (Invitrogen). Labelled probes were purified using the PCR purification kit from Qiagen. Probes were pooled, and 60 μg Cot1 DNA and 100 μg tRNA were added. This mixture was then concentrated in a speed vacuum and adjusted to a volume of 15 μl containing 3.4×SSC with 0.3% SDS final concentration. After a short denaturation step, the mixture was prehybridised for 4 h at 37°C to block repetitive sequences. The prehybridised DNA was then transferred to the chip and hybridised for 16 h under a coverslip in a humidity chamber (Genemachine). Slides were washed for 5 minutes in 2×SSC with 0.03% SDS at 65°C, 5 minutes in 1×SSC at 65°C, and 5 minutes in 0.2×SSC. The slides were then dried by centrifugation.

Following hybridisation, arrays were scanned in the Cy3 and Cy5 channels (595 nm a 685 nm, respectively) using an Arrayworks reader and software (Applied Precision). Using the DataBridge software (GeneScan Europe AG), the ratios of the Cy3 and Cy5 signals were normalised relative to the signals from the reference BAC clones from the X chromosome, with the average ratio of all reference clones set to 1, as described previously.

**RESULTS**

**Validation of the array CGH with a 4 Mb SOX9 deletion case**

To validate the sensitivity and reproducibility of our CGH and microarraying protocols, we used DNA from patient 1 with a large SOX9 deletion previously identified by us, who had been described only in preliminary form. FISH analysis with the 2.4 Mb SOX9 spanning YAC 946E12 revealed a complete absence of the corresponding region on one of the two chromosomes 17 in this patient (fig 2A). The centromeric end of the human insert in this YAC was sequenced and mapped to position 70 669 708 (November 2002 freeze, human genome browser at UCSC; http://genome.ucsc.edu); its telomeric end is 250 kb distal to SOX9 (fig 2C). Haplotyping analyses performed with the Thermosequenase II Dye Terminator Cycle Sequencing kit (Amersham Pharmacia) and analysed on an ABI Prism 310. Conserved sequence elements were identified by BLAST searches of human sequences against the Takifugu sequence release 3 on the (Takife)Gu website (http://tfgu.hgmp.mrc.ac.uk), and against mouse sequences at the NCBI BLAST site (http://www.ncbi.nlm.nih.gov/BLAST). PipMaker analyses were performed at http://bio.cse.psu.edu/pipmaker.
Figure 2  A large interstitial deletion spanning SOX9 in patient 1 (A) FISH with YAC 946 E12 on metaphase spread showing complete absence of signal on the del(17) chromosome. (B) Haplotype analysis of the family of patient 1, showing hemizygosity for paternal alleles of microsatellite markers either side of SOX9 in the patient. (C) Relative positions of polymorphic markers used, with their physical distances from the tip of the short arm of chromosome 17 (November 2002 freeze, human genome browser at UCSC). All markers were used for somatic cell hybrid analysis (see text); markers informative for the haplotype analysis shown in B are underlined. YAC-CEN and YAC-TEL denote the centromeric and telomeric insert end, respectively, of YAC 946E12, used in (A). The minimum and maximum extent of the patient 1 deletion is indicated on the right.
To independently determine the size of the deletion, we isolated the deleted chromosome 17 from a normal chromosome 17 in a mouse/human somatic cell hybrid. By STS content mapping of the del(17) hybrid using the markers shown in fig 2C, the centromeric and telomeric end of the deletion could be assigned to the intervals D17S940/D17S949 and D17S1352, respectively, in agreement with the FISH and haplotype data (data not shown).

The DNA of patient 1 was then used for array CGH. The positions of the chromosome 17 BAC and PAC clones used for the microarray are shown in fig 3A. Whereas the Cy5/Cy3 ratios for the 17 BAC/PAC clones were all ~1.0 in control hybridisations with normal genomic DNA used as test DNA (fig 3B), the ratios for the 12 BAC/PAC clones from the patient 1 deletion were consistently reduced to ~0.5 if patient 1’s DNA was used as test DNA (fig 3C).

Array CGH detects a large deletion in the SOX9 5’ control region in patient 2

Having established the CGH DNA chip protocol with the deletion case (patient 1), we used this protocol to screen a sample of 11 non-translocation campomelic cases, six of which were classified as typical CD/ACD cases according to the criteria of Mansour et al., the other five presenting only with some of the features. For all cases, we had excluded a SOX9 coding region mutation by sequencing, and a SOX9 deletion by Southern dosage blots or quantitative PCR. A single sample gave a CGH result indicative of a deletion 5’ to SOX9, showing a reduced signal for seven contiguous clones, with all Cy5/Cy3 ratios <0.8 (fig 3D). This indicates a minimum size for the deletion of 0.9 Mb (fig 3A). This DNA sample derived from patient 2, a 46,XY sex reversed patient with the acampomelic form of CD.

The patient 2 deletion is 1.5 Mb in size

To define the extent of the deletion more precisely, the two copies of chromosome 17 of patient 2 were separated in mouse/human somatic cell hybrids. Fragment analysis using microsatellite marker D17S808, which lies centromeric to the deletion (fig 2C) showed that of the two alleles of 145 bp and 159 bp present in patient 2’s DNA (fig 4A, lane 3), hybrid 1 (H1) showed only the 145 bp allele (lane 1), while hybrid 19 (H19) showed only the 159 bp allele (lane 2). As shown below, hybrid 1 contains the del(17) chromosome, hybrid 19 the normal chromosome 17. Genotyping of this patient’s mother (fig 4A, lane 4) revealed that the deleted chromosome in hybrid 1 is of maternal origin (DNA from the father was not available). As the mother was heterozygous and patient 2 hemizygous for the marker D17S949 that lies within the deletion (fig 2C), the deletion must have arisen de novo (data not shown).

STS markers, developed from the available genomic sequence, were then used for STS content mapping of DNA from hybrid 1, using DNA from hybrid 19 as positive and mouse DNA as negative controls. Starting from the information provided by the CGH data (fig 3D), the centromeric end of the deletion could be narrowed down to a 3 kb interval flanked by STS markers RP17 and RP18, which typed positive and negative for hybrid 1, respectively (fig 4B, top left). The telomeric end of the deletion could similarly be assigned to a 2.5 kb interval defined by STS markers RP13 and RP14, which again typed negative and positive for hybrid 1, respectively (fig 4B, top right). Subsequently, the forward primer for RP17 and the reverse primer for RP14 were extended to a length of 25 nucleotides each and were used in long range PCR, yielding a 4.2 kb fragment with DNA from hybrid 1 and from the total genomic DNA of patient 2, but not from a control DNA or with mouse DNA (fig 4B, bottom). This PCR fragment enabled us to sequence directly across the deletion (fig 5B). The centromeric end of the deletion lies within BAC 118F13 (not used for CGH), between bases 139,568 and 139,572 (GenBank accession no. AC005242), 1,869 bp upstream of the SOX9 transcription start site. The telomeric deletion breakpoint lies between bases 4054 and 4058 of BAC 432M11 (GenBank accession no. AC006448), 379,769–71 bp upstream of SOX9. (The central CAA nucleotides in fig 5B could derive from either clone; see fig 5A). The patient 2 deletion is thus 1,489,662 bp in size.

The patient 2 deletion removes two sequence elements conserved between human, mouse, and fish

The patient 2 deletion spares the five potential regulatory sequence elements E1–E5, which are highly conserved between human, mouse, and Takifugu, located from 28 kb to 290 kb upstream of SOX9 (fig 5C). These elements are all about 100 bp in size and show sequence identities of 67–80% and 81–95% between human and Takifugu, and between human and mouse, respectively. A BLAST search of the 1.5 Mb sequence deleted in patient 2 against the recent Takifugu sequence release 3 disclosed two conserved sequence stretches, each with a highly conserved core of ~120 bp with 76–79% identity. These sequence elements, designated Eb1 and Eb2, are 395 kb and 611 kb, respectively, from human...
Figure 4  Somatic cell hybrid analysis of the patient 2 deletion. (A) Microsatellite analysis. The electropherogram displays allele sizes for microsatellite marker D17S808 from dyelabelled nucleic acid fragments of somatic cell hybrid 1 with the paternal chromosome 17 of patient 2 (lane 1), hybrid 19 with the paternal chromosome 17 of patient 2 (lane 2), patient 2 (lane 3), and patient 2’s mother (lane 4), separated on an ABI Prism 310 genetic analyser and analysed by the Genotyper software (version 3.7). (B) Typing of STS markers flanking the centromeric and telomeric deletion breakpoint. Note that STS markers RP18 and RP13 within the deleted region resulted in no amplification for hybrid 1. Bottom, deletion spanning long range PCR with primer pair RP17 F-Long and RP14 R-Long showed amplification of a 4.2 kb-fragment for hybrid 1 and patient 2, but not for a normal control DNA and for mouse DNA. P2, patient 2; C, normal human control; H1, hybrid 1; H19, hybrid 19; M, size marker; Mo, mouse.

SOX9 (fig 5C), and 38 kb and 56 kb from a second Takifugu SOX9 parologue that had not been available at our previous analysis, but are absent from the previously characterised Takifugu SOX9 locus. Eb1 and Eb2 are also conserved in mouse. BLAST and PipMaker analyses of the deleted human sequence with the corresponding mouse sequence, using a minimum size of 100 bp and a minimum sequence identity of 90%, identified an additional 10 conserved sequence blocks (R1–R10 in fig 5C) (sequences available on request).

DISCUSSION

Two patients with large deletions in the SOX9 region are described in the present report. One deletion, found in patient 1, extends to both sides of the SOX9 gene. Patient 1 is the only second case with a molecularly confirmed complete deletion of SOX9; the first case has been described in an XX female infant with an interstitial deletion 17q23.3-q24.3, who died 4 days after birth.26 The fact that no symptoms other than those typically seen in CD were observed in patient 1 may be attributable to the fact that only 10 genes are located within the 4.0 Mb minimum, and 15 genes within the 5.1 Mb maximum deletion interval. Both SOX9 deletion cases provide the strongest evidence so far that CD is a SOX9 haploinsufficiency disorder.

The second deletion, found in patient 2, is 1.5 Mb in size and lies entirely within the 2 Mb region 5′ to SOX9 that is devoid of any protein coding gene. Analysis of the patient 2 deletion reveals that the centromeric breakpoint lies within a partial LINE-1 repeat (L1, fig 5C). Unequal homologous recombination between LINE-1 elements has been described as a mutational mechanism generating interstitial deletions. However, the telomeric breakpoint of the patient 2 deletion lies 400 bp from another partial LINE-1 element. Several interstitial deletions have been described where a LINE-1 repeat is present on one side and an unrelated sequence on the other side of the deletion breakpoint, as in the present case.22–24 The involvement of LINE-1 is probably fortuitous in all these cases.

An indication as to which mechanism might have created the patient 2 deletion is given by the 3 bp homology, CAA, at the deletion junction (fig 5A). Short homologies of 2–6 bp have been observed at the junctions of both small and larger sized deletions in the human genome (Woods-Samuels et al25 and references therein). Bullock et al26 observed 2–3 bp homologies at the excision points for SV40 in cultured rat cells and showed that the sequences at these crossover points were associated with eukaryotic topoisomerase I cleavage sites in vitro. In fact, one of the crossover points had a CAA homology, followed by a T and an A residue, respectively, as in fig 5A, and was shown to be cut at each site by topoisomerase I after the T residue opposite the central A in the CAA sequence. As topoisomerase I is known to also catalyse the ligation of nonhomologous DNA in vitro,27,28 this enzyme might have been involved in the creation of the patient 2 deletion by mechanisms similar to those described.25–27 Whether the nearly identical sequences either side of the centromeric and telomeric deletion junction (underlined in fig 5A) contributed to the recombination event remains unclear.

The 1.5 Mb patient 2 deletion provides the first strong evidence for the existence of cis regulatory elements more than 380 kb 5′ to human SOX9. The presence of such distantly upstream elements is also supported by the CD translocation breakpoints t(5;17) and t(17;22), respectively located 324–346 kb and 932–966 kb 5′ to SOX9 (corrected coordinates), which retain elements E1–E5 in front of SOX9 (fig 5C). The effect of these two translocation breakpoints, the most distant from SOX9 presently known, may also be explained, however, by position effects due to juxtaposition of silencers or of heterochromatin.29 They are therefore no solid evidence for such long distance cis regulatory elements. On the other hand, there is some evidence for a very distant
Figure 5  Sequence of the deletion junction and precise extent of the patient 2 deletion. (A) The normal centromeric (Cen) and telomeric (Tel) sequences are shown aligned with the deletion junction sequence (DJ). Homology between the normal and junction sequence is indicated by bold lettering. The junctional CAA homology is set off from the rest of the sequence. Nearly identical sequences 5 (12/15 identities) and 3 (8/11 identities) of the centromeric and telomeric deletion junction are underlined by straight and dashed lines, respectively. (B) The electropherogram shows the sequence across the deletion breakpoint. The sequences terminate within BAC clones RP11–118F13 and RP11–423M11 as indicated. (C) Details of the deleted region, drawn to scale. The centromeric deletion breakpoint is within a partial LINE-1 (L1) repeat, while the telomeric breakpoint is close to a sequence across the deletion breakpoint. The sequences terminate within BAC clones RP11–118F13 and RP11–423M11 as indicated. (C) Details of the centromeric deletion breakpoint

cis acting element for Sox9 expression in the mouse. The dominant mouse mutant Ods carries a 150 kb deletion 1 Mb 5' to Sox9 that leads to upregulated Sox9 expression in fetal gonads of XX mice, causing XX sex reversal. This deletion is thought to remove a regulatory element that mediates repression of Sox9 expression in XX fetal gonads, a repression antagonised by SRY in XY embryos, according to the repressor model of sex determination in mammals. The human sequence corresponding to the Ods deletion is located 1.2–1.4 Mb upstream of SOX9 (fig 5C). Because the 1.5 Mb deletion in patient 2 occurred on an XY background and caused male to female sex reversal, the deletion is, unfortunately, not informative with respect to XX—that is, female to male sex reversal.

Within the patient 2 deletion, two sequence elements, Eb1 and Eb2, conserved between human, mouse, and fish, and an additional 10 sequence elements, R1–R10, conserved only between human and mouse, were identified (fig 5C). One or more of these sequence elements may function as general or tissue specific regulators for SOX9. It is tempting to speculate that one of these elements is actually responsible for expression of SOX9 in testis, as a human YAC transgene containing 350 kb of SOX9 5' flanking sequence failed to show expression in the testes of transgenic mice, and as testis development did not occur in patient 2. Functional tests in transgenic mice will show which, if any, of the elements are involved in regulating SOX9 expression.

Patient 2 is the only case from the sample studied in which a deletion was detected. The other 10 cases may have a deletion below the detection limit of the array CGH protocol used, which we estimate to be about half a clone size. As the sizes of the BAC and PAC clones used range from 85 kb to 203 kb, we would have detected a deletion ≥50–100 kb in size in the other campomelic cases studied. These cases, therefore, have either a smaller sized deletion in the SOX9 5' flanking 1 Mb region, a point mutation or microdeletion in a control element, or a mutation elsewhere in the SOX9 region. Alternatively, as some of the cases did not fulfill the minimum set of criteria for a classical CD or ACD case, the causative mutation may lie in a gene other than SOX9.

The two deletion cases presented here, both of which have a 46,XY karyotype, highlight the aspect of variable expressivity with regard to XY sex reversal seen in CD. Whereas patient 1 with deletion of the entire SOX9 region was born as a boy with normal male external genitalia, patient 2 with the upstream deletion that leaves the SOX9 ORF intact is a completely sex reversed girl with normal female external genitalia (no gonad histologies are available for either patient). Likewise, identical point mutations in the SOX9 coding region can cause XY sex reversal in one case, and no sex reversal in another. Contributions from the genetic background and/or environmental factors, or stochastic fluctuation of SOX9 transcription levels, can be invoked to explain how reduced SOX9 dosage can lead to such highly divergent sexual phenotypes.

Whereas no correlation exists between SOX9 dosage and XY sex reversal, there appears to be some correlation between severity of the skeletal phenotype and survival time. We have previously noted that CD patients with translocations tend to have a longer life expectancy than patients with mutations in

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the SOX9 coding region, and that these two classes of CD mutations also differ with respect to the eponymous feature, camptomelia, which is present in only half of the translocation cases but in over 90% of the SOX9 ORF mutation cases. A recent study also shows that surviving cases of CD are frequently translocation cases. Patient 2 resembles the surviving case, and she has acampomelia. The milder phenotype in all these cases most probably results from residual activity of the SOX9 allele on the affected chromosome. The translocation and the 5′ deletion in patient 2 thus represent hypomorphic alleles of SOX9 rather than complete loss of function alleles.

In conclusion, the present study provides strong evidence for the existence of cis acting regulatory elements more than 380 kb upstream of SOX9. It also demonstrates the usefulness of array CGH for the detection of deletions in a large genomic region such as the 5′ control region of SOX9. The deletion detection limit could be reduced to 40 kb or less, if cosmid of array CGH for the detection of deletions in a large genomic region as is frequently the case in severe disorders such as CD.

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REFERENCES