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Report

Fine Mapping of Chromosome 17 Translocation Breakpoints ⩾900 Kb Upstream of *SOX9* in Acampomelic Campomelic Dysplasia and a Mild, Familial Skeletal Dysplasia

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Previously, our group reported a five-generation family in which a balanced t(13;17) translocation is associated with a spectrum of skeletal abnormalities, including Robin sequence, hypoplastic scapulae, and a missing pair of ribs. Using polymerase chain reaction (PCR) with chromosome-specific markers to analyze DNA from somatic cell hybrids containing the derivative translocation chromosomes, we narrowed the breakpoint on each chromosome. Subsequent sequencing of PCR products spanning the breakpoints identified the breaks precisely. The chromosome 17 breakpoint maps ~932 kb upstream of the sex-determining region Y (SRY)–related high-mobility group box gene (*SOX9*) within a noncoding transcript represented by two IMAGE cDNA clones. A growing number of reports have implicated chromosome 17 breakpoints at a distance of up to 1 Mb from *SOX9* in some cases of campomelic dysplasia (CD). Although this multigeneration family has a disorder that shares some features with CD, their phenotype is significantly milder than any reported cases of (nonmosaic) CD. Therefore, this case may represent an etiologically distinct skeletal dysplasia or may be an extremely mild familial example of CD, caused by the most proximal translocation breakpoint from *SOX9* reported to date. In addition, we have refined the breakpoint in an acampomelic CD case described elsewhere and have found that it lies ~900 kb upstream of *SOX9*.

Campomelic dysplasia (CD [MIM 114290]) is a dominant, frequently lethal skeletal dysplasia syndrome whose primary features include angular bowing and shortening of the limbs, hypoplastic scapulae, missing ribs, a narrow thorax, tracheal abnormalities, respiratory distress, club feet, dysmorphic features, and, in a majority of affected XY individuals, partial-to-complete sex reversal (Houston et al. 1983; Mansour et al. 1995). Most cases of CD are caused by mutations in *SOX9*, the gene encoding a member of the sex-determining region Y (SRY)–related high-mobility group box family of transcription factors located on human chromosome 17q24.3-q25.1 (Foster et al. 1994; Wagner et al. 1994). *SRY* is the gene encoding Y-linked testis-determining factor (Sinclair et al. 1990; Koopman et al. 1991), and mutations in *SRY* also cause XY sex reversal (Berta et al. 1990; Jager et al. 1990). *SOX9* is downstream of—and may be a direct target of—*SRY* in the male sex-determination cascade (Knower et al. 2003). Interestingly, ectopic expression of *Sox9* can induce testis development in the absence of *Sry* in XX transgenic mice (Vidal et al. 2001). During chondrogenesis, the *SOX9* gene targets include the collagen-encoding genes *Col2a1* and *Col11a2* as well as *CD-Rap* (Bell et al. 1997; Lefebvre et al. 1997; Ng et al. 1997; Bridgewater et al. 1998; Xie et al. 1999). Therefore, *SOX9* expression and targets correlate with the tissues primarily affected in CD—namely, cartilage and testis. *SOX9* mutations identified in cases of CD are inactivating, and, in two reported cases, the entire *SOX9* locus was deleted, which confirms that CD is due to haploinsufficiency of *SOX9* (Olney et al. 1999; Scherer et al. 1999; Pop et al. 2004). The dose-dependent role of *SOX9* in sex determination is further supported by a case in which duplication of the region containing *SOX9* caused sex reversal in an

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XX infant (Huang et al. 1999). Moreover, it has been shown that dimerization of SOX9 protein is necessary for transactivation of targets in chondrocytes but not in testis, suggesting that different tissues should have varying sensitivities to *SOX9* dose (Bernard et al. 2003; Sock et al. 2003). However, there is no correlation between the specific type and location of *SOX9* mutation and the sexual phenotype; at least two mutations have been identified that cause sex reversal in one XY individual but not in a second XY patient with an identical mutation (Kwok et al. 1995; Cameron et al. 1996; Meyer et al. 1997). This suggests that phenotypic variation is heavily influenced by the level of expression from the unaffected *SOX9* allele and/or by stochastic factors. The only genotype-phenotype association for which a case can be made is the correlation between survival beyond infancy and retention of some residual function by the mutant *SOX9* allele (Meyer et al. 1997).

Perhaps the most interesting aspect of CD is the growing number of reports in which the associated lesion is not a *SOX9* mutation but is instead a chromosome 17 rearrangement breakpoint located some distance from *SOX9* (Maraia et al. 1991; Young et al. 1992; Foster et al. 1994; Wagner et al. 1994; Ninomiya et al. 1995, 1996; Wirth et al. 1996; Savarirayan and Bankier 1998; Pfeifer et al. 1999; Mansour et al. 2002; Offiah et al. 2002). These breakpoints range from tens to hundreds of kilobases upstream and downstream of *SOX9* (Velagaleti et al. 2005 [in this issue]) and show no apparent bias toward chromosomal translocation partner, and, although affected individuals with these breakpoints have, in general, milder phenotypes and longer survival than those with classic CD, they still exhibit XY sex reversal and infant mortality. On the basis of these translocation breakpoints, it has been speculated that regulatory elements for *SOX9* can be located as far as 1 Mb upstream of *SOX9* (Wunderle et al. 1998; Pfeifer et al. 1999; Bagheri-Fam et al. 2001; Pop et al. 2004).

We report here the precise mapping of chromosome 17 translocation breakpoints nearly 1 Mb upstream of *SOX9* in two previously described patients with CD or CD-like skeletal dysplasia. Patient MS was an XY male with acampomelic CD (and a survival time of 6 years) and a balanced t(17;22) translocation whose chromosome 17 breakpoint was previously reported to be 932–966 kb upstream of *SOX9* (Wagner et al. 1997; Pfeifer et al. 1999; Pop et al. 2004). We have reanalyzed this breakpoint and have found that it lies 900.2 kb upstream of the *SOX9* transcriptional start and within 1 kb of a breakpoint reported by Velagaleti et al. (2005). Family F carries a balanced t(13;17) translocation and exhibits a mild and slightly variable skeletal dysplasia, consisting primarily of Robin-type cleft palate, a missing pair of ribs, and hypoplastic scapulae, in members of at least five generations (Stalker and Zori 1997; Stalker et al. 2001). We have mapped the chromosome 17 breakpoint in family F at 931.8 kb upstream of *SOX9*. This case represents the translocation breakpoint most proximal from *SOX9* reported to date that is associated with a skeletal dysplasia phenotype. The breakpoint interrupts a noncoding, spliced, and polyadenylated transcript of unknown function that is expressed in testis.

Family F: Sequencing of the *SOX9* coding region and splice sites from family F revealed no mutations. To better understand the skeletal dysplasia syndrome in family F, we chose to map precisely the breakpoints in their balanced t(13;17) translocation. As a first step, human-mouse somatic cell hybrids were commercially made using patient leukocytes and were screened for their human chromosome content (GMP Genetics). Using a reiterative, PCR-based process of typing hybrids that contain the derivative translocated chromosomes for chromosome-specific markers, we mapped the chromosome 13 breakpoint to an interval of 413 bp and the chromosome 17 breakpoint to an interval of 288 bp. Primer sequences for nonpublic mapping markers are available in table A1 (online only). A subset of the mapping results is shown in tables 1 and 2. We then generated cross-breakpoint PCR products by use of primers from the mapping markers that fell closest to the breakpoints (indicated in tables 1 and 2). These PCR products were

Table 1

Partial Translocation Breakpoint Mapping Results for Chromosome 13 Markers in Family F, t(13;17)(q22.1;q22.3)

	Finding in Family F[a]			Marker Coordinates[b]	
		Translocated			
Marker	Normal	der(13)	der(17)	Start	End
13 gap 23	+	+	−	88356686	88356925
13 gap 27.3	+	+	−	88399721	88400054
13-28.7	+	+	−	88414493	88414730
13-30.1	+	+	−	88428157	88428377
13 gap 31.6	+	+	−	88442841	88443080
13-16	+	+	−	88459861	88460075
13-5550	+	+	−	88466013	88466273
13-11111[c]	+	+	−	88471622	88471904
13-401[c]	+	−	+	88472317	88472522
up-gap 13	+	−	+	88473289	88473618
down-gap 13	+	−	+	88474944	88475209
13-33	+	−	+	88476702	88476935
D13S1818[d]	+	−	+	88492889	88493108
D13S767[d]	+	−	+	88916866	88917030
D13S1190[d]	+	−	+	90149068	90149317
SHGC 16473[d]	+	−	+	90729432	90729566
D13S71[d]	+	−	+	93571723	93571797
D13S796[d]	+	−	+	106686966	106687128

[a] + = marker is present; − = marker is absent.
[b] NCBI chromosome 13 sequence, build 35.1.
[c] Markers flanking the breakpoint.
[d] Publicly available markers.

Table 2

Partial Translocation Breakpoint Mapping Results for Chromosome 17 Markers in Family F, t(13;17)(q22.1;q22.3), and Patient MS, t(17;22)

	Finding in Family F[a]			Finding in Patient MS	Marker Coordinates[b]	
		Translocated				
Marker	Normal	der(13)	der(17)	der(22)	Start	End
D17S1350[c]	+	−	+	−	66600358	66600540
RH65556[c]	+	−	+	−	66670860	66671047
RP-20	+	−	+	−	66674098	66674497
17 gap 20530	+	−	+	−	66691461	66691719
17-2200	+	−	+	−	66694306	66694588
17-4551[d]	+	−	+	−	66696681	66696862
DP-29[d]	+	+	−	−	66697150	66697256
17-28370	+	+	−	−	66698729	66699105
17-34220	+	+	−	−	66705191	66705450
17 gap 41070	+	+	−	−	66712140	66712377
C11BP1	+	+	−	−	66718464	66718796
C11BP2	+	+	−	−	66724488	66724712
C11BP3	+	+	−	−	66726775	66727010
C11BP5	+	+	−	−	66727576	66727788
C11BP6	+	+	−	−	66728086	66728286
C11BP7[e]	+	+	−	−	66728349	66728522
C11BP8[e]	+	+	−	+	66728730	66728894
C11BP4	+	+	−	+	66728921	66729178
17 gap 600	+	+	−	+	66731367	66731624
17 gap 12	+	+	−	+	66790865	66791168

[a] + = marker is present; − = marker is absent.
[b] NCBI chromosome 17 sequence, build 35.1.
[c] Publicly available markers.
[d] Markers flanking the family F breakpoint.
[e] Markers flanking the patient MS breakpoint.

of identical size and sequence when generated from somatic cell hybrid DNA or patient DNA, indicating that no extraneous rearrangements were present in the hybrids that would complicate our analysis (not shown). Direct sequencing of the der(13) PCR product revealed 342 nt of chromosome 13 sequence followed immediately by 165 nt of chromosome 17 sequence (fig. 1). The der(17) product contained small deletions of 8 nt from chromosome 17 and 13 nt from chromosome 13 that were adjacent to the breakpoints, perhaps as a result of degradation of the broken ends during the translocation event (fig. 1). There was no apparent homology

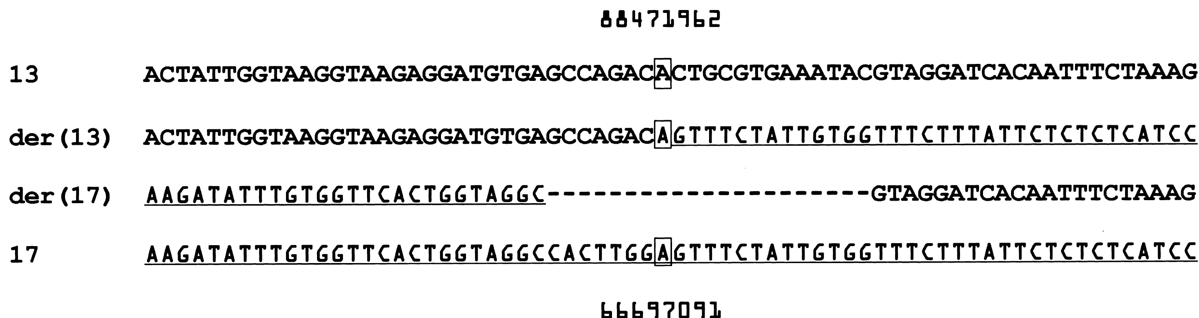
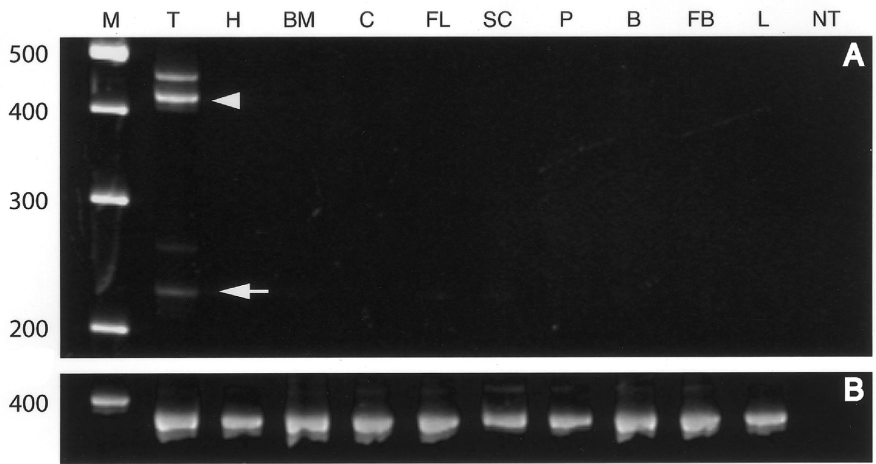
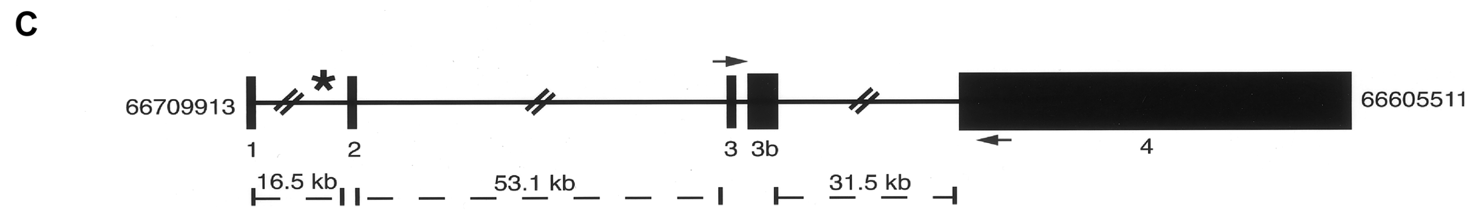
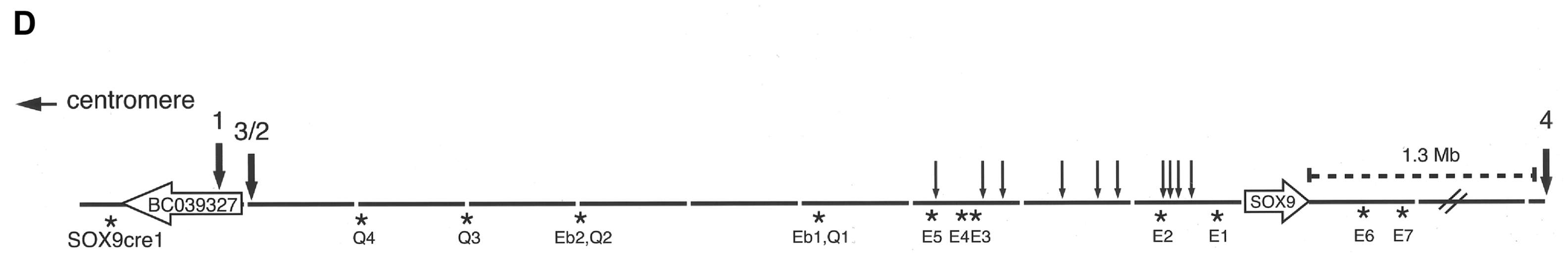


Figure 1 Family F translocation breakpoint sequences. Partial sequence of PCR products spanning the translocation breakpoints on the der(13) and der(17) t(13;17) chromosomes. Normal sequences from chromosomes 13 and 17 are shown above and below the breakpoint sequences, respectively, along with the sequence coordinate corresponding to the breakpoint. The chromosome 13 sequence is in bold, and the chromosome 17 sequence is underlined. The boxed A nucleotide at the breakpoint junction cannot be assigned to either chromosome sequence. The der(17) chromosome contains small deletions of 8 nt from chromosome 17 and 13 nt from chromosome 13 (*dashes*).


M
T
H
BM
C
FL
SC
P
B
FB
L
NT
500
400
300
200
A
400
B


C
66709913
66605511
1
2
3
3b
4
16.5 kb
53.1 kb
31.5 kb


D
centromere
1
3/2
4
1.3 Mb
BC039327
SOX9
SOX9cre1
Q4
Q3
Eb2,Q2
Eb1,Q1
E5
E4E3
E2
E1
E6
E7

between the breakpoint sequences that might have contributed to the translocation mechanism. The chromosome 13 break, therefore, falls at nucleotide 88471962 in the National Center for Biotechnology Information (NCBI) chromosome 13 reference sequence (build 35.1), and the chromosome 17 break falls at nucleotide 66697091 in the NCBI chromosome 17 reference sequence, 931.8 kb upstream of the *SOX9* transcriptional start.

After mapping the translocation breakpoints of family F, we examined the sequence in the vicinity of each breakpoint for evidence of transcript(s) that might be interrupted or otherwise affected by the translocation. The chromosome 13 breakpoint fell within the 5′ EST read sequence of IMAGE clone 6619359, which was retired from the UniGene database (it was formerly cluster Hs.508345) shortly after we mapped this breakpoint. The chromosome 17 breakpoint falls within the first intron of a transcript that has been independently isolated as a cDNA at least twice: once from testis RNA and once from an RNA pool that included testis. One corresponding cDNA clone, IMAGE clone 5267277 (GenBank accession number BC039327), has been fully sequenced and was used as the reference sequence for our further investigations. A BLAST search of the BC039327 sequence against the NCBI human chromosome sequence database allowed us to determine an intron/exon structure (shown in fig. 2*C*) and to visually examine the putative intron/exon boundaries for splice-donor and splice-acceptor sites, all of which were present (not shown). This clone contains four exons spaced over ~104 kb of genomic DNA, a polyadenylation signal, and a poly-A tail. RT-PCR spanning exons 1–3 (not shown) and exons 3–4 (fig. 2*A*) generated products of the expected size from testis RNA, confirming that this transcript is both expressed in testis and spliced. Heart, bone marrow, cerebellum, fetal liver, spinal cord, placenta, adult brain, fetal brain, lymphocytes (fig. 2*A*), and adult liver (not shown) were all negative for sequence BC039327 by RT-PCR but were positive for the housekeeping gene hypoxanthine phosphoribosyl transferase (*HPRT*) (fig. 2*B*). The RT-PCR reactions for BC039327 exons 3–4 generated four distinct bands, including the expected band at ~224 bp (arrow in fig. 2*A*). Sequencing of the most prominent product (arrowhead in fig. 2*A*) revealed the expected sequence of exons 3–4 and a stretch of 185 nt that is not found in BC039327. This 185-nt sequence fell precisely at the junction of exons 3 and 4, suggesting that it corresponded to an alternatively spliced exon. A BLAST search confirmed that this 185-nt sequence is present on chromosome 17, between BC039327 exons 3 and 4 and in the appropriate orientation, which confirms that this sequence represents an additional alternative exon (exon 3b) (fig. 2*C*) for the transcript represented by the BC039327 sequence. Homology searches identified exon 3b as containing an L1-type long interspersed nuclear element repeat. The two other bands generated by RT-PCR were not sequenced but are assumed to represent additional alternative splice forms of this transcript.

In silico translation of the BC039327 sequence (with or without exon 3b) revealed no ORFs >69 amino acids and no methionine initiation codon surrounded by a Kozak consensus sequence (Kozak 1987). Therefore, this transcript is unlikely to encode a protein and may instead represent a functional RNA. A search for homologues in other species revealed only tiny fragments of homology within exon 4: 85 nt of 89% identity and 24 nt of 100% identity with mouse sequence from (syntenic) chromosome 11, as well as similar results with rat, chimpanzee, and gorilla sequences (data not shown). No mouse ESTs showed any homology to the BC039327 sequence. Therefore, this transcript appears to be specific to humans.

Patient MS: Previous analysis indicated that *SOX9* sequence from patient MS was normal. Our early STS mapping results for family F suggested that their breakpoint must be very close to that reported for patient MS

Figure 2 RT-PCR and genomic organization of the BC039327 transcript. *A,* RT-PCR of BC039327 exons 3–4. *B,* Control RT-PCR for the housekeeping transcript *HPRT.* Lanes are marked as follows: M = 100-bp marker; T = testis; H = heart; BM = bone marrow; C = cerebellum; FL = fetal liver; SC = spinal cord; P = placenta; B = brain; FB = fetal brain; L = lymphocytes; NT = no template, control. Sizes of marker bands are shown (*left*). *C,* Genomic organization of the BC039327 locus on 17q. The centromere is to the right of the figure. Exons are represented by blackened rectangles, and exon numbers are indicated directly beneath them. The approximate positions of primers used for RT-PCR in *A* are shown as arrows (not to scale). Intron spans are indicated (*dashed lines*), and the size of each intron is given in kb. The position of the family F translocation breakpoint is designated by an asterisk (*). The chromosome 17 sequence coordinates (NCBI build 35.1) for the first and last (non–poly-A tail) nucleotides of the BC039327 sequence are indicated at the left and right of the locus diagram, respectively. *D,* Overview of the genomic region surrounding *SOX9* on 17q. Line segments equal ~100 kb of genomic sequence each. CD translocation breakpoints summarized by Pfeifer et al. (1999) are indicated by thin vertical arrows, located on the basis of their closest possible position with respect to *SOX9.* Breakpoint positions reported in the present study and by Velagaleti et al. (2005) are indicated by thick vertical arrows and are labeled as follows: 1 = family F; 2 = patient MS; 3 = patient 1 in Velagaleti et al.; 4 = patient 2 in Velagaleti et al. Approximate positions of highly conserved (human, mouse, and *Takifugu*) sequence elements described elsewhere are indicated with asterisks (*) and are labeled as follows: E1–E7, Bagheri-Fam et al. (2001); Q1–Q4, Qin et al. (2004); Eb1 and Eb2, Pop et al. (2004); and SOX9cre1, Velagaleti et al. (2005). The mammalian elements R1–R10 reported by Pop et al. (2004) are not shown.

by Pfeifer et al. (1999, case 11). We used the same PCR-based strategy and both the existing and the newly designed chromosome 17–specific primer sets on somatic cell hybrid DNA containing the der(22) chromosome to map the breakpoint of patient MS to the 208-bp interval between chromosome 17 nucleotides 66728522–66728730 (NCBI build 35.1). This breakpoint is ~32 kb telomeric to the family F break (table 2) and 900.2 kb 5′ of the *SOX9* transcriptional start. There are no known or predicted transcripts in the vicinity of this breakpoint. The chromosome 22 breakpoint in this patient is located within heterochromatin and could not be mapped.

CD usually presents as an isolated de novo condition with a severe phenotype leading to death in infancy. Familial cases are rare and appear to be due to parental mosaicism for a *SOX9* mutation. In such cases, the parent may be completely unaffected and yet have more than one affected child as a result of germline mosaicism (Shafai 1976; Mellows et al. 1980; Winter et al. 1985; Mansour et al. 1995, 2002; Cameron et al. 1996; McDowall et al. 1999). In other cases, the parent is mildly affected and is diagnosed with CD only after the birth of a severely affected child (Thurmon et al. 1973; Lynch et al. 1993; Mansour et al. 2002; Savarirayan et al. 2003). In at least one such case, a mutation was confirmed in both the mother and the child (Mansour et al. 2002). Although the mild phenotype of the parents of patients with CD is assumed to be due to somatic mosaicism, this has not been proven. To the best of our knowledge, there are no reports of familial CD extending to three or more generations. Affected individuals that survive into adulthood show variable expression of the classic CD features (Mansour et al. 2002; Offiah et al. 2002; Savarirayan et al. 2003). As they age, these individuals demonstrate short stature, progressive scoliosis, and, frequently, hearing loss. Therefore, long-term survivors of CD have a set of phenotypic features that is consistent and recognizable (Mansour et al. 2002).

The skeletal syndrome in family F shares several features with CD (Stalker and Zori 1997; Stalker et al. 2005; Unger 2005). Indeed, Robin sequence, hypoplastic scapulae, and 11 pairs of ribs—the primary features in family F—are nearly universal in both mild and severe forms of CD. Family F also has minor facial dysmorphism consisting of flat face, broad nasal bridge, micrognathia, and hypertelorism that is consistent with CD. However, many of the other features of CD are not present in this family: stature and lifespan are normal; there is no bowing of the long bones and no clubbing of the feet; pelvic bones, spine, and epiphyses are normal; and there is no evidence of sex reversal. The respiratory difficulty reported for one affected child (Stalker and Zori 1997) was believed by the family to be caused by a large cleft palate, although medical records were not available, so this finding is purely anecdotal and cannot be confirmed. Most notably, the scoliosis and hearing loss that are nearly ubiquitous in long-term survivors of CD, as reported by Mansour et al. (2002), are not seen in family F. Therefore, although family F displays a skeletal dysplasia reminiscent of CD, it remains unclear whether this syndrome is unique or is, in fact, an extremely mild form of CD (Stalker et al. 2005; Unger 2005).

Although CD is usually caused by mutation of *SOX9,* 14 cases to date have been reported in which the only identifiable lesion is a chromosomal rearrangement involving the long arm of chromosome 17. *SOX9* is found at 17q24.3-25.1, and the chromosomal breakpoints associated with CD occur as far as 1 Mb upstream (or >1 Mb downstream, as reported by Velagaleti et al. [2005]) of *SOX9.* The data suggest that *SOX9* possesses regulatory elements that are extraordinarily distant, extending 1 Mb upstream and >1 Mb downstream of the *SOX9* coding region. This inference is supported by data from the *Odd Sex* mouse, in which insertion of a transgene driven by the dopachrome tautomerase (Dct) promoter 980 kb upstream of murine *Sox9* leads to inappropriate expression of *Sox9* in XX gonads and to XX sex reversal (Bishop et al. 2000; Qin et al. 2004), demonstrating that sequence ~1 Mb upstream of *Sox9* is able to affect *Sox9* expression. Supporting evidence for long-distance regulation of *SOX9* is also provided by a CD case with a deletion extending from 380 kb to 1.869 Mb 5′ of *SOX9* (Pop et al. 2004). Studies of human *SOX9* 5′ sequence in mouse have shown that 350 kb of upstream sequence was sufficient to drive normal-level expression of a *SOX9:lacZ* reporter gene in cartilage but not in testis, whereas 88 kb of upstream sequence gave little to no *lacZ* expression except in neuroectoderm, in which expression was delayed (Wunderle et al. 1998). Comparison of sequence between human, mouse, and *Takifugu* revealed five conserved sequence elements in the 290 kb 5′ of *SOX9,* many of which are separated from *SOX9* by CD translocations (Bagheri-Fam et al. 2001). Qin et al. (2004) reported four sequence elements, 390–800 kb upstream of *SOX9,* that are conserved between human, mouse, and chicken. Pop et al. (2004) examined 1.5 Mb of sequence upstream of *SOX9* and identified two widely conserved sequence elements 395 kb and 611 kb upstream of human *SOX9,* as well as 10 sequence elements farther upstream that are conserved only between human and mouse. It has been suggested that one or more of these elements may be responsible for testis-specific regulation of *SOX9,* because all 12 are deleted in a CD case with a 1.5-Mb 17q interstitial deletion and sex reversal, whereas 350 kb of *SOX9* 5′ sequence is insufficient for expression of a *SOX9* reporter in testis (Wunderle et al. 1998; Pop et al. 2004). However, 7 of the 10 more-centromeric elements are separated from *SOX9* in both of the translocation cases addressed in

the present study, of which one exhibits acampomelic CD without sex reversal and the other displays a mild, familial skeletal dysplasia with no sex reversal. Therefore, the role of the identified conserved sequence elements in regulation of *SOX9* remains to be determined. However, Velagaleti et al. (2005) provide evidence that a conserved sequence element ~1.1 Mb upstream of *SOX9* is in close physical proximity to the *SOX9* locus in interphase chromosomes, supporting the possibility of *SOX9* regulation by distant regulatory elements. Such long-range elements have been demonstrated for the genes *POU3F4* (de Kok et al. 1996), *Sonic hedgehog* (Lettice et al. 2003), *MAF* (Jamieson et al. 2002), and *DACH* (Nobrega et al. 2003). A summary of the reported CD translocation breakpoints and widely conserved potential *SOX9* regulatory elements is provided in figure 2*D*. It is interesting to note that, although conserved sequence elements have been identified throughout the 1 Mb of sequence upstream of *SOX9*, there is a marked absence of reported CD translocation breakpoints in the region 300–900 kb upstream.

Although there are no protein-coding genes within the 1 Mb of sequence 5′ of *SOX9*, the family F breakpoint interrupts a noncoding transcript that extends over ~104 kb of genomic sequence and is spliced and polyadenylated. Because of the CD-like phenotype in this family, it is possible that the interrupted transcript is involved in regulation of *SOX9* as a functional RNA. Since CD is due to loss of *SOX9* function, the potential role of this transcript is most likely that of a positive regulator. Of 11 tissues tested by RT-PCR, the BC039327 sequence was detected only in testis. Although this result is consistent with a potential role in *SOX9* regulation, there is no testis phenotype in family F. It may be that a single copy of BC039327 is sufficient for normal *SOX9* regulation in testis but not in chondrocytes, thereby generating the skeletal-specific phenotype. Alternatively, this transcript may play a role in chondrogenesis that is unrelated to *SOX9*. It should be noted that several other noncoding transcripts of unknown function have been identified in the region around *SOX9*, some of which are also expressed in testis (Ninomiya et al. 1996; Pfeifer et al. 1999; Bagheri-Fam et al. 2001). Alternatively, the disruption of the BC039327 transcript may be coincidental, and the syndrome in this family may be caused by misregulation of *SOX9* due to separation from one or more distant regulatory elements. The fact that this translocation breakpoint is the farthest one (upstream) from *SOX9* reported to date suggests that it leaves the majority of the *SOX9* regulatory region intact, perhaps accounting for the mild phenotype in this family. The CD translocations in patient MS (in the present report) and patient 1 (in Velagaleti et al. [2005]) are 32 kb telomeric to the family F breakpoint and, remarkably, are 1 kb apart at most. These are the only CD breakpoints reported to fall within 600 kb of the family F breakpoint. The difference in phenotypic severity and diagnosis between family F and these patients suggests that the intervening 32 kb of sequence may be important for *SOX9* regulation, although no potential regulatory elements have been reported in this interval. Wirth et al. (1996) were able to demonstrate expression from both *SOX9* alleles in a patient with CD and a t(13;17) translocation; however, this experiment used lymphoblast RNA, which may not reflect expression in the developmentally relevant tissues—fetal testis and chondrocytes. Indeed, it is essentially impossible to obtain patient samples that would allow meaningful analysis of *SOX9* expression and regulation. Therefore, the elucidation of how the DNA sequence surrounding *SOX9* contributes to its regulation must, for the present, continue to rely on circumstantial evidence of CD translocation breakpoints and comparative sequence analyses. Such studies will not only add to our understanding of how one of the most important factors in development, *SOX9*, is regulated but will also shed light on the roles of gene-poor "desert" regions in the genome.

Acknowledgments

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Electronic-Database Information

The accession number and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for IMAGE clone 5267277 [accession number BC039327])
NCBI, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CD)

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