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ORIGINAL INVESTIGATION

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SNTG1, the gene encoding γ1-syntrophin: a candidate gene for idiopathic scoliosis

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Abstract Idiopathic scoliosis (IS) affects approximately 2%–3% of the population and has a heritable component. The genetics of this disorder are complex. Here, we describe a family in which a pericentric inversion of chromosome 8 co-segregates with IS. We have used fluorescence in situ hybridization to identify cloned DNAs that span the breakpoints on the two arms of the chromosome. We have identified a bacterial artificial chromosome (BAC) of 150 kb that crosses the q-arm breakpoint and a BAC of 120 kb that crosses the p-arm breakpoint. The complete genomic DNA sequence of these BACs has been analyzed to identify candidate genes and to localize further the precise breakpoints. This has revealed that the p-arm break does not interrupt any known gene and occurs in a region of highly repetitive sequence elements. On the q-arm, the break occurs between exons 10 and 11 of the γ-1 syntrophin (SNTG1) gene. Syntrophins are a group of cytoplasmic peripheral membrane proteins that associate directly with dystrophin, the Duchenne muscular dystrophy gene; γ1-syntrophin has been shown to be a neuronal cell-specific protein. Mutational analysis of SNTG1 exons in 152 sporadic IS patients has revealed a 6-bp deletion in exon 10 of SNTG1 in one patient and a 2-bp insertion/deletion mutation occurring in a polypyrimidine tract of intronic sequence 20 bases upstream of the SNTG1 exon 5 splice site in two patients. These changes were not seen in a screen of 480 control chromosomes. Genomic DNAs from seven affected individuals within the family of a patient carrying the 6-bp deletion were typed to determine whether the alteration co-segregated with IS. The deletion was only observed in five out of these seven individuals. Thus, although genetic heterogeneity or multiple alleles cannot be ruled out, the 6-bp deletion does not consistently co-segregate with the disease in this family.

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Introduction

Scoliosis is a prevalent abnormality of the vertebral column in which patients develop lateral curvature of the spine. By definition, this curvature is greater than 10° as measured by the Cobb method (Cobb 1948). Scoliosis often occurs secondary to bone, neurologic, or neuromuscular disease, e.g., in osteogenesis imperfecta (Sillence et al. 1986), Charcot-Marie-Tooth disease (Hensinger and MacEwen 1976; Walker et al. 1994) or the muscular dystrophies (Do 2002; Yamashita et al. 2001). However, the most common form is idiopathic scoliosis (IS) in which no preexisting cause for the curvature can be determined. IS is the most common pediatric spinal deformity seen by physicians, affecting 2%–3% of the population worldwide, with onset usually in adolescence (Lonstein 1994). This prevalence decreases, with increasing severity, to 0.2%–0.3% for curves requiring treatment. About one tenth of the latter group will require surgical correction. There is also a well-described gender bias with increasing disease severity. The ratio of girls to boys with curves of less than 10° is near equal but increases sharply

to 7:1 when only curves under treatment are considered (Rogala et al. 1978).

A genetic basis for IS (MIM 181800) has been widely described (Beals 1973; Harrington 1977), and epidemiological studies indicate that 20%–30% of IS cases have a familial component (Lonstein 1994; Weinstein 1991). Twin studies have consistently shown a high concordance rate. In a meta-analysis of existing literature combined with their own data, Kesling and Reinker (1997) showed 73% concordance in monozygotic twins compared with 36% concordance in dizygotic twins. A second study of 21 twin pairs found concordance in 92.3% of monozygotic twins compared to 62.5% of dizygotic twins (Inoue et al. 1998). Even though the genetic contribution to IS has been confirmed and accepted, the inheritance pattern is unclear. Most studies suggest that IS is a complex disease with a multi-factorial pattern of inheritance (Riseborough and Wynne-Davies 1973; Berven et al. 1997). Nevertheless, some families have been described that exhibit clearer cut patterns of inheritance, and several genetic linkage studies have localized putative susceptibility loci. Wise et al. (2000) localized IS susceptibility loci to chromosomes 6p, 10q, and 18q. Other studies have identified putative loci on chromosome 19p (Chan et al. 2002) and chromosome 17p11 (Salehi et al. 2002). Despite these advances, no mutated gene has yet been identified as causing or contributing to human IS. This may in part be because genetic linkage or association studies are complicated by the poorly defined spectrum of phenotypes, polygenic inheritance, a high rate of phenocopies, and locus heterogeneity.

An alternative strategy to the conventional positional cloning approach is to identify one or more chromosomal alterations in affected individuals. The hypothesis is that the chromosomal break marks the exact location of the culprit gene causing disease (Bugge et al. 2000; Collins 1995). Historically, balanced chromosomal rearrangements have proved extremely useful in the identification of Mendelian loci. Two examples are the genes that cause type 1 neurofibromatosis (Fountain et al. 1989) and polycystic kidney disease (European Polycystic Kidney Disease Consortium 1994). This strategy carries the caveat that chromosomal rearrangements may occur coincident with the disease but have no causative role. However, it is relevant to point out that, in a large epidemiological study relating cytogenetic abnormalities to early developmental disorders in young children, Warburton (1991) concluded that about two thirds of observed chromosomal abnormalities were probably causally related to these early onset disorders. With this in mind, we set out to explore a previous report that described the co-segregation of a balanced chromosomal rearrangement with scoliosis in one small pedigree (Szappanos et al. 1997). In the current report, we show that this rearrangement is a pericentric inversion of chromosome 8 and that the q-arm break occurs in the γ1-syntrophin gene (SNTG1; Piluso et al. 2000). Syntrophins are a group of cytoplasmic peripheral membrane proteins that associate directly with dystrophin, the product of the Duchenne muscular dystrophy gene. Mutational analysis of SNTG1 in our collection of IS patients identified two alterations that were not found in a parallel screen of 480 unaffected patient chromosomes. However, expansion of this screen to other family members did not show simple co-segregation of scoliosis with the alterations.

Materials and methods

Cytogenetic methods

Fluorescence in situ hybridization (FISH) for sublocalization was performed by using probes derived from yeast and bacterial artificial chromosomes (YACs and BACs). Inter-Alu polymerase chain reaction (PCR) products were prepared from YAC DNAs by previously published procedures (Lengauer et al. 1992). In addition, BACs were purified and used as individual cloned probes (see below). Approximately 20 metaphase chromosome spreads derived from lymphoblastoid cell lines from affected and normal individuals were examined for each hybridization.

Each DNA probe (2 μg) was biotin-labeled via nick-translation and 100–150 ng was used in hybridizations to metaphase chromosome spreads (Lengauer et al. 1992). Human Cot-1 DNA (Roche; 5–20 μg) was added per hybridization reaction to reduce non-specific hybridization. The metaphase slides were incubated with fluorescein-isothiocyanate (FITC), and signals were amplified via incubation in FITC-conjugated goat anti-avidin D antibodies (Vector Laboratories; 5 μg/ml). Chromosomes were counterstained in a final wash of 200 ng/ml 4,6-diamino-2-phenylindole and 200 ng/ml propidium iodide. Visualization of FISH signals was accomplished by using a Zeiss Axioplan 2 microscope, and images were captured by using an ISIS in situ imaging system (Metasystems, Belmont, Mass.).

YAC and BAC isolation

YAC clones were obtained from a library of human YACs (Brownstein et al. 1989). BAC clones were obtained from the Childrens Hospital Oakland Research Institute (Oakland, Calif.). YAC DNA was isolated by means of a modified protocol for yeast DNA mini-preparation as described by Lee (1992). BAC and P1 artificial chromosome DNAs were isolated by using the Qiaprep Spin Miniprep Kit (Qiagen).

Patient and control collections

Informed consent, health history, and consent for release of records were obtained from all participating individuals. Whole blood (10–20 ml) was collected from each participant by venipuncture. A diagnosis of IS was made from medical history and examination of spinal radiographs. A minimum measured Cobb angle of 15° was required for an individual to be scored as affected. All patients were of European origin. DNA was isolated from whole blood by using an isolation kit as recommended by the manufacturer (Wizard Genomic DNA Purification Kit; Promega, Madison, Wis.). Control DNAs were from the CEPH (Centre d'Etude Polymorphisme Humain).

PCR amplification

SNTG1 exons were amplified from genomic DNA by means of the oligonucleotide primers listed in online (electronic supplementary material, Table 2). All amplifications were carried out in a PCR Express Thermal Cycler (Hybaid, Middlesex, UK). Reactions were

performed in 50 µl containing 1.5 mM $MgCl_2$, 500 mM KCl, 0.25 mM each dNTP, 1.0 pmol each primer, 0.5 U *Taq* polymerase (PE, Norwalk, Conn.), and 150 ng patient DNA. Amplification conditions involved touchdown PCR with 1 cycle at 94°C for 30 s, 58°C for 30 s, 68°C for 30 s, and then reduction of the annealing temperature by 1°C per cycle to 53°C, followed by 30 cycles of 94°C for 30 s, 53°C for 30 s, and 68°C for 30 s.

Mutational analysis

Mutation screening was performed by denaturing high-performance liquid chromatography (DHPLC) on a DHPLC WAVE machine (Transgenomics, San Jose, Calif.) under conditions recommended by WaveMaker v3.3.3 software. PCR-amplified products were resolved and purified from 1% agarose gels with the QIAquick gel purification kit (Qiagen, Hilden, Germany), and DNA was sequenced with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, Calif.). Sequencing products were separated by electrophoresis on an ABI PRISM 377 DNA Sequencer (Applied Biosystems). Supplementary information is available at http://hg.wustl.edu/lovett/projects/scoliosis/index.html.

Results

IS and a chromosomal rearrangement were originally observed in a father and son of a small eastern European family who reported that the deceased grandfather was also affected (Szappanos et al. 1997). Our cytogenetic analysis of lymphoblastoid cells lines derived from these two patients (ISHunSR and ISHunJR, father and son, respectively) revealed the presence of a pericentric inversion on chromosome 8 occurring between 8p23.2 and 8q11.21 (Fig. 1B, C). The same analysis revealed normal chromosomes in the unaffected grandmother, consistent with the possible inheritance of the inversion from the grandfather. A search of the most common chromosomal rearrangement polymorphisms (Kleczkowska et al. 1987; Tierney et al. 1984) indicated that pericentric inversions in these regions of chromosome 8 are uncommon (Jacobs et al. 1974; Boyd et al. 1994).

To refine the location of each breakpoint, we employed FISH to metaphase chromosomes from the cell line ISHunSR. We selected YACs and BACs that mapped to the approximate chromosomal break regions to identify regions above and below each breakpoint. We then refined the location of each break by *in silico* walking through the reference sequence to pick BAC clones that fell within the candidate interval.

The p-arm break

Figure 2A shows a physical map of the YACs and BACs that we used to localize the p-arm break, relative to the reference human genome physical map. Two YACs (882_D_6 and 807_A_1) were identified by FISH as mapping above the break (see Fig. 2B for a representative FISH result). Because 882_D_6 maps telomerically to 807_A_1, it is not shown in Fig. 2A. Four YACs (910_F_12, 879_F_11, 967_C_11, and 792_A_6) were identified as mapping below the p-arm break (see Fig. 2C for a representative hybridization). Three YACs (832_G_12, 843_H_5, and 841_F_8) and one BAC (RP11–29A2) were identified as spanning the p-arm break (see Fig. 2D,E). The hybridization results showed clearly that the genomic clones hybridized to both arms of the inversion chromosome, but only to the p-arm of the wild-type chromosome 8.

The genomic DNA sequences within BAC RP11–29A2 are predominantly comprised of highly repetitive sequence blocks (small and long interspersed nuclear elements, long terminal repeats, and simple class and low complexity family of repeats). These large repetitive sequence tracts and the lack of single-copy sequence "handles" made the identification, molecular cloning, and DNA sequencing of the exact breakpoint particularly difficult. Analysis of the

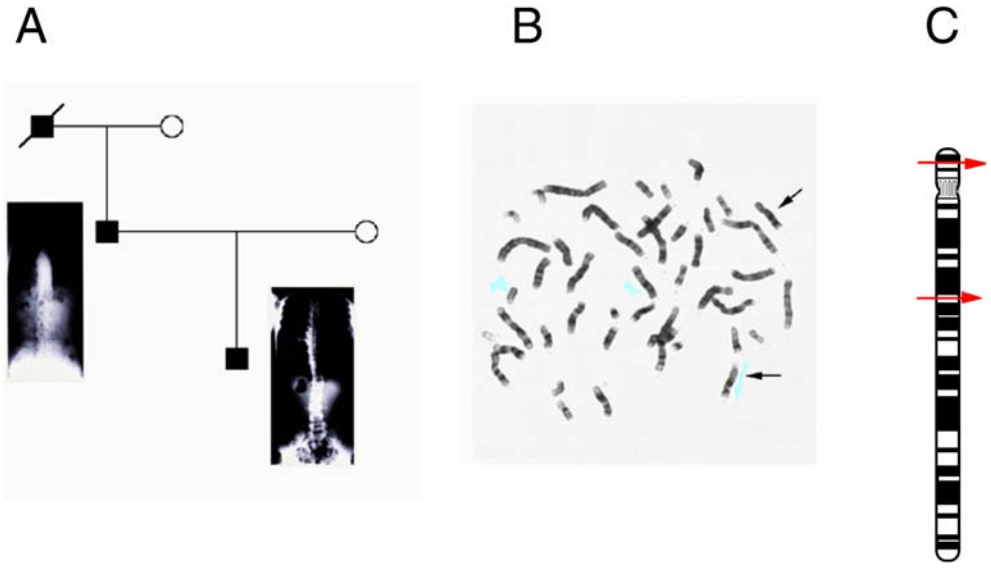

Fig. 1A–C Family with scoliosis used in this study. **A** Father and son (X-rays presented) have a common chromosomal rearrangement identified as a pericentric inversion of chromosome 8. **B** G-banded metaphase of father. *Arrows* Wild-type chromosome 8 and chromosome 8 with pericentric inversion. **C** Ideogram showing the pericientric inversion and breakpoints (*red arrows*)

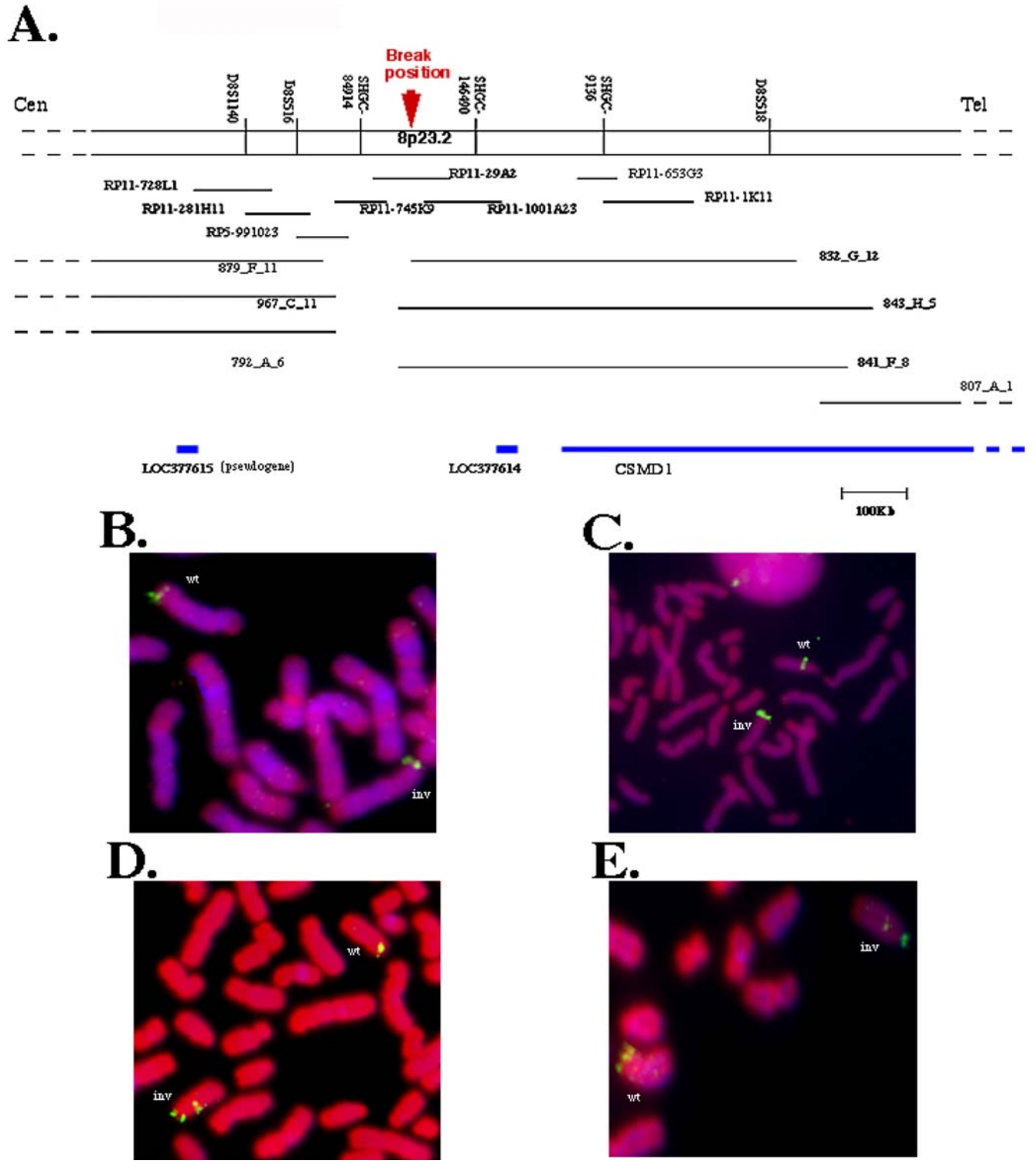

Fig. 2 **A** Physical map of the YACs and BACs used to localize the p-arm break, relative to the reference human genome physical map. **B–E** FISH results obtained by using YAC 882_D_6 (**B**) that maps above the break and YAC 967_C_11 (**C**) that maps below the break and by using BAC RP11–29A2 (**D**) and YAC 843_H_5 (**E**) that cross the break

predicted genes in the 380-kb vicinity of RP11–29A2 revealed only one major gene: the gene CSMD1, which encodes the CUB and sushi multiple domains protein 1, lies approximately 263 kb away from the ends of BAC RP11–29A2 and is transcribed away from the breakpoint region. We were unable to detect the expression of CSMD1 in control and patient lymphoblastoid cell lines by reverse transcription/PCR (RT-PCR; data not shown); therefore, we could not determine whether expression was affected. Whereas we cannot rule out a position effect

upon this flanking gene (or upon genes even further away), it is clear from our FISH results that this gene is not directly broken by the inversion. A predicted gene (LOC377614) that shares similarity to the 60 S ribosomal protein L10 is also present approximately 102 kb from the ends of BAC RP11–29A2. Using RT-PCR (data not shown), we could not detect any change in expression levels of LOC377614 in control and patient lymphoblastoid cell lines, indicating the apparent absence of a position effect on the expression of this gene.

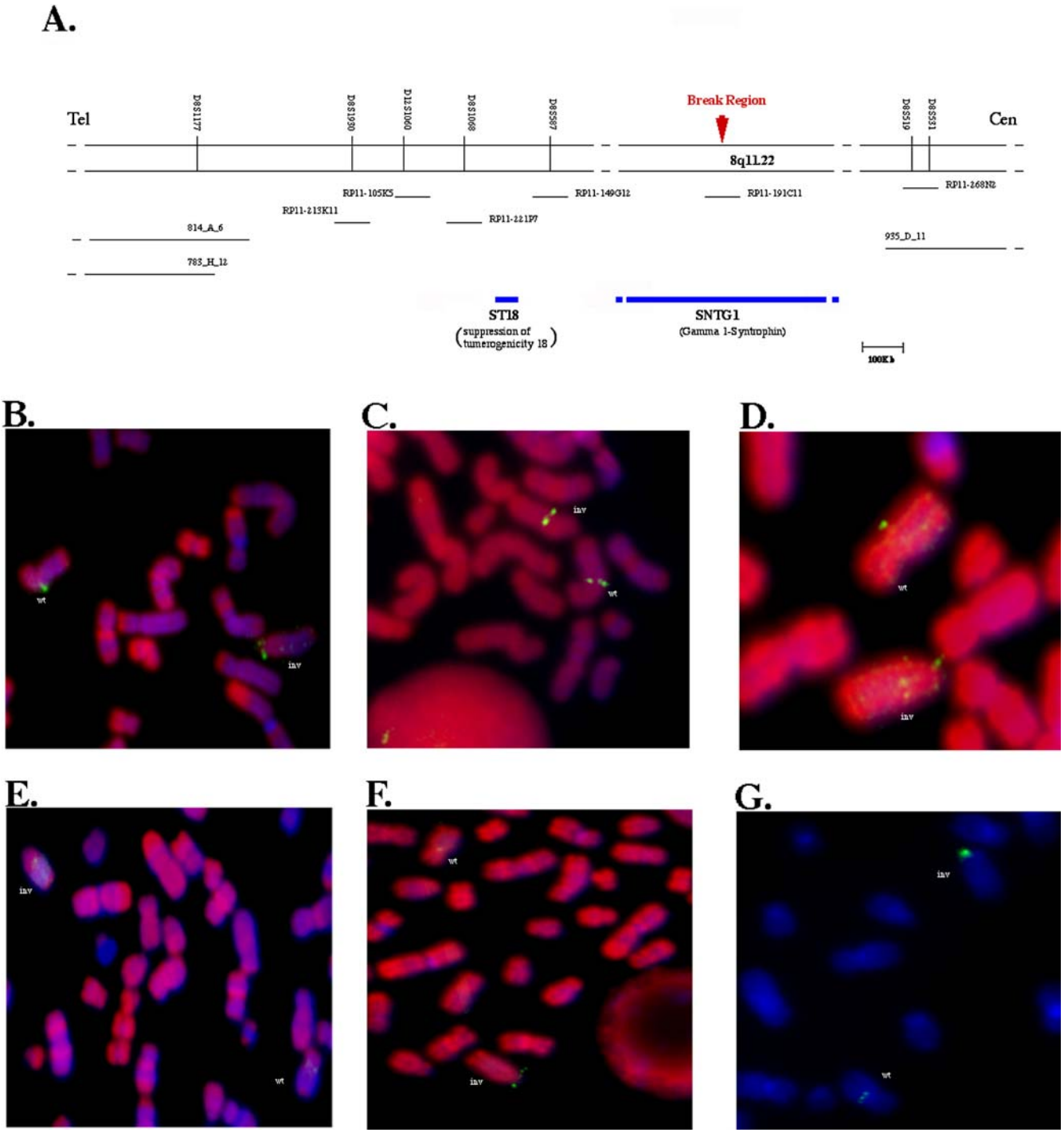

Fig. 3 **A** Physical map of the YACs and BACs used to localize the q-arm break, relative to the reference human genome physical map. **B–D** FISH results obtained by using BAC clone RP11–268N2 (**B**) that maps above the break, BAC clone RP11–149G12 (**C**) that maps below the break, and BAC clone RP11–191C11 that crosses the breakpoint (**D**). **E–G** Localization of the break within the SNTG1 gene by using PCR products from the SNTG1 sequence as FISH probes. 3'Region1 maps below break (**E**). A combination of 5'Region1, 5'Region2 and 5'region3 maps above the break (**F**), as does 3'Region2 alone (**G**)

The q-arm breakpoint

Chromosome 8q was analyzed by FISH by using YAC and BAC clones that had been previously localized to the 8q11.21 region (derived from the Whitehead Institute/MIT Center for Genome Research and National Center for Biotechnology Information Web Sites). The large insert clones in this region are shown in Fig. 3A, and the results of FISH with these various probes are shown in Fig. 3B–D. Hybridizations with clones that map above the break (e.g., BAC clone 268N2, YAC clone 935D11) are shown in Fig. 3B. FISH with clones that map below the break (e.g., BAC clone 149G12, YAC clone 783H12) are shown in Fig. 3C. One BAC clone (RP11–191C11) was identified that crossed the break on chromosome 8q (Fig. 3D). The reference human genome DNA sequence indicates that this BAC contains part of SNTG1.

SNTG1 is broken by the inversion

We confirmed the presence of SNTG1 sequences in the BAC clone by designing primers for regions spanning the gene and testing the BAC by PCR. A list of primers and the regions that they amplify are given in the online supplementary material (electronic supplementary material, Table 1). The BAC included SNTG1 sequences from the intron between exons 9 and 10 up to the intron between exons 12 and 13 (Fig. 4). All other regions of SNTG1, either upstream or downstream, failed to amplify the BAC. We further localized the q-arm breakpoint by using PCR products amplified from the SNTG1 gene sequence as probes for FISH (Fig. 4). Amplified regions were selected because of the absence of repetitive sequences and ranged from 1.8 kb to 3.2 kb in size (electronic supplementary material, Table 1). By using these probes, the break was further localized to a region from the intron between exons 9 and 10 up to the intron between exons 10 and 11 (see Figs. 3E–G, 4). Having localized the break to a region of 32 kb, we next sought to clone and sequence the breakpoint. However, in a similar manner to that observed with the p-arm, the 8q break region is plagued with highly repetitive blocks creating the same localization and cloning difficulties. Therefore, in the absence of patient DNA sequences in this region, we cannot rule out the possibility that a deletion or rearrangement of sequences has occurred at the breakpoints. However, it is clear that the q-arm break does indeed break within a known gene, viz., SNTG1.

Mutation screening of SNTG1

In order to assess whether the SNTG1 gene is more broadly relevant to IS, we next searched for mutations in this candidate gene in DNAs collected from 150 IS patients. Primers were designed to amplify each of the 20 SNTG1 exon sequences and to include intron-exon boundaries (electronic supplementary material, Table 2). Genomic DNAs derived from 150 sporadic and familial IS patients of European origin were used as a template to amplify each exon sequence. Exons from the IS patients were analyzed by DHPLC to identify potential variants within the IS cases. Any putative variants were directly DNA-sequenced to confirm the exact nature of the observed DHPLC change. Two variants were identified by this method: a deletion in exon 10 in a single patient, and a two base insertion/deletion within the intron adjacent to exon 5 in two patients. The deletion in exon 10 of patient 16-1 encompassed six bases in one allele immediately adjacent to the 3' exon 10 splice junction (electronic supplementary material, Fig. 1). This change was not detected in a parallel screen of 480 normal chromosomes. The deletion itself reconstructs a canonical splice donor site. Therefore, splicing may not be disrupted, but one definite consequence of this alteration would be a deletion of two amino acids within the PDZ domain. Either way, the consequences of this deletion in patients are difficult to assess. All available information on SNTG1 indicates that it is expressed exclusively in neurons. We

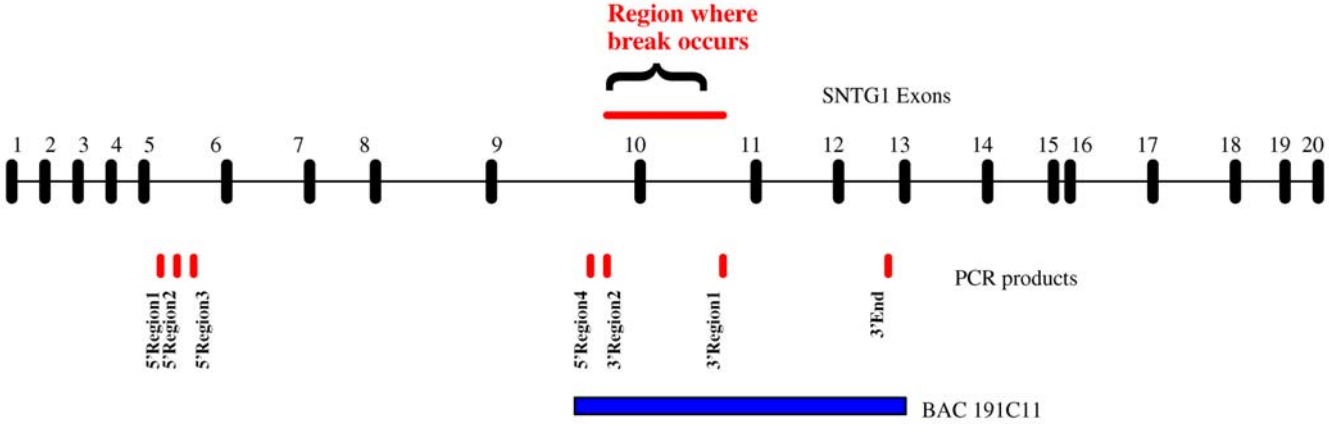

Fig. 4 Schematic representation of SNTG1; exon numbers are indicated. Position of BAC RP11–191C11 relative to the gene is shown. *Red lines* Amplified SNTG1 sequences used to ensure the presence of the SNTG1 sequence within the BAC and employed as FISH probes to further narrow down the break region

were unable to detect its expression in lymphoblastoid cell lines (data not shown) and thus could not ascertain, by RNA or cDNA sequence analysis, whether the SNTG1 mRNA was altered by this change. Future studies will explore the effects of this simple deletion of two amino acids upon SNTG1 function in neuronal cells.

The variation in intron 4/exon 5 of patient YY and patient ZZ was a two-base insertion/deletion event (the deletion of a single G and replacement by two Ts) in one allele in the intron sequence upstream of exon 5 (electronic supplementary material, Fig. 1). Again, this change was not found in a screen of 240 control CEPH DNA samples. This change alters a polypyrimidine tract that lies 10 bp upstream of the splice site that is believed to affect splicing fidelity (Coolidge et al. 1997). The tract represents an important part of the *cis*-acting sequences required for eukaryotic pre-mRNA splicing; alterations in this sequence may affect this process by preventing correct spliceosome assembly (Ruskin and Green 1985; Frendewey and Keller 1985). The change that we have observed alters the sequence from $T_3G_1T_{10}$ to a longer consecutive polypyrimidine T_{15} tract and thus may increase the usage of the flanking splice site and potentially affect SNTG1 splice forms. Again, because SNTG1 expression appears to be restricted to neural lineages, we could not check this hypothesis by direct measurement of SNTG1 splice forms in human patients.

Testing for co-segregation

The individual carrying the 6-bp SNTG1 exon 10 alteration is a member of a family with many individuals affected by IS. As is shown Fig. 5, this pedigree is not ideal for validating identified variants. We confirmed the affected status of the mother by radiographic analysis; however, the deceased father was reported by the family to have some form of spinal deformity, raising the possibility that disease alleles in the children could have been inherited from either parent. Nevertheless, we typed all ascertained affected individuals (n=7, of which two were monozygotic twins) to determine whether the alteration co-segregated with IS. Five of the seven affected individuals, including the twins, carried the deletion. The two who did not were individual IS 16-7 and his daughter IS 16-5. Thus, whereas genetic heterogeneity or multiple alleles cannot be ruled out, the 6-bp deletion does not consistently co-segregate with the disease in this family.

Discussion

In this report, we have shown that a pericentric inversion of chromosome 8, which is present in two related individuals with IS, physically breaks SNTG1. The p-arm break occurs within a region that is particularly rich in repetitive elements (and is gene poor). Our available evidence argues against a position effect of the break upon flanking p-arm genes. First, the two genes that exist in the general locale of the p-arm break are several hundreds of kilobases away, and position effects over such large distances would be exceptional in human genetics. However, precedents do exist for relatively long range position effects in humans, e.g., cases of aniridia where breakpoints at 100 kb and 125 kb downstream of the *PAX6* gene affect its expression (Fantes et al. 1995). Second, our semi-quantitative RT-PCR experiments (data not shown) failed to show any abundance changes for these genes. Nevertheless, we cannot completely rule out the involvement of sequences on 8p in the observed phenotype.

The most obvious effect of the inversion is the disruption of the SNTG1 gene on chromosome 8q. The critical questions that this observation raises are (1) is the apparent co-segregation of IS with this inversion merely coincidence, (2) does SNTG1 have broader relevance to IS in the general population, and (3) how could this neuronal protein possibly be functionally connected to curvature of the spine?

As noted above, epidemiological information (Warburton 1991) indicates that about two thirds of balanced cytogenetic abnormalities are probably causally related to early developmental disorders in young children. Presumably, this is an underestimate when later onset disorders such as IS are included into the phenotypic spectrum. The chromosomal inversion that we have observed in this study places what appears to be transcriptionally silent DNA adjacent to the broken SNTG1 gene. The expected outcome of this would be haploinsufficiency for the SNTG1 protein. The most direct means of testing whether this gene is relevant to IS are

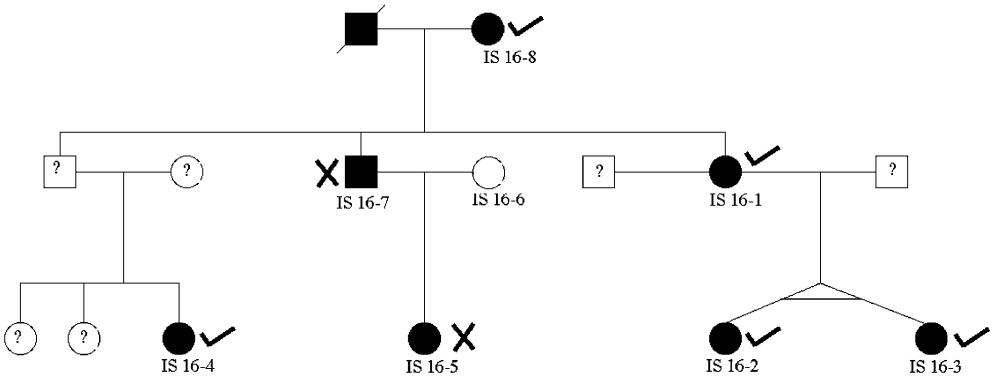

Fig. 5 Pedigree of family IS 16. *Tick* Deletion detected in family member indicated, *cross* deletion not detected

either to construct an animal model by gene knockout methods or to assess whether alterations in SNTG1 are more prevalent in IS patients than in controls. We are not aware of any mouse *sntg1* knockout strains, but even if these were constructed, a quadrepedal model for IS might be uninformative for the reasons described further below. In this study, we did mutationally analyze SNTG1 in 150 IS patients and found three patients corresponding to two possible mutations. These alteratons were not detected in 240 wild-type DNAs. However, when we followed this up by typing additional IS family members in a small pedigree that carried one of the variants, the change in SNTG1 did not co-segregate with the disease in all affected children. This may be because alleles of two different scoliosis loci were segregating in this pedigree. Clearly, additional studies need to be conducted on more straightforward IS pedigrees before this issue can be resolved. It will also be of interest to conduct association studies with markers across this region to ascertain the possible contribution of SNTG1 to sporadic and familial IS. In this regard, it is interesting to note that a recent report (Justice et al 2002) listed several IS genetic linkage peaks, including one on chromosome 8q in the general vicinity of SNTG1.

How could a neuronal-specific protein influence curvature of the spine? The underlying pathogenesis of IS is unclear. Although muscle, bone, and nerve tissues are all implicated, one prevailing theory is that the primary lesion is a neurological defect causing abnormal central nervous system (CNS) processing leading to anomalies in the developing spine (Lowe et al. 2000; Maguire et al. 1993; Yamada et al. 1984). These are believed to lead to changes in posture and secondary effects on muscle and bone tissue physiology attributable to alterations in force distribution (Manzoni and Miele 2002; Yamada et al. 1984). A large body of evidence supports this notion. For example, animal models of scoliosis suggest that postural control (and bipedalism) play an important part in the development of IS. Pinealectomized chickens have consistently been shown to develop scoliosis (Thillard 1959; Dubousset et al. 1983), whereas pinealectomized rats and hamsters do not (O'Kelly et al. 1999). However, if rats are first rendered bipedal and then pinealectomized, they develop scoliosis (Machida et al. 1999). Similarly, destruction of the reticular formation of the brain stem in bipedal rats also produces scoliosis (Yamada et al. 1984). The correlation between damage to the CNS and scoliosis has been shown in cynomolgus monkeys when dorsal spine nerve roots are severed (Pincott et al. 1984). In addition, several studies in humans describe the association between decreased standing stability and development of scoliosis (Byl and Gray 1993; Chen et al. 1998; Nault et al. 2002; Burwell et al. 1992). Thus, disruption of postural equilibrium may play an important role in progressive spinal curvature. The known biology of SNTG1 fits well with this theory. Syntrophins exist as five isoforms (α1, β1, β2, γ1, and γ2) encoded by five distinct genes. They are known to be parts of the dystrophin-associated protein complex and interact directly with the C terminus of dystrophin. The various syntrophin genes are expressed in a restricted tissue-specific pattern (Ahn et al. 1996). γ1-Syntrophin is specifically localized to neuronal cells (Piluso et al. 2000). In the brain, it is predominantly expressed in the Purkinje neurons of the cerebellum and also in the pyramidal neurons of the hippocampus and the cortex (Hogan et al. 2001). One function of the cerebellum is to coordinate movement and maintain balance (Womack and Khodakhah, 2002). Thus, SNTG1 is expressed in precisely those areas of the brain that have been suggested to affect postural control and by implication IS. Taken together, the observations that the SNTG1 gene is predominantly expressed in the cerebellum, hippocampus and cortex, that it is disrupted by an inversion in one IS pedigree, and that it has sequence alterations in some sporadic IS patients suggest that it represents an interesting candidate for involvement in IS.

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