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Identification of two alternative fusion genes, SYT-SSX1 and SYT-SSX2, in t(X;18)(p11.2;q11.2)-positive synovial sarcomas

B.de Leeuw*, M.Balemans, D.Olde Weghuis and A.Geurts van Kessel

Department of Human Genetics, University Hospital, PO Box 9101, 6500 HB Nijmegen, The Netherlands

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Synovial sarcomas are soft tissue tumors that occur mainly in adolescents and young adults. The chromosomal translocation (X;18)(p11.2;q11.2) is found in the majority of these sarcomas (1) and, as such, is thought to play a causative role in tumor formation. The occurrence of two related but distinct breakpoints in Xp11.2 has been reported by us and others using tumor-derived somatic cell hybrids and metaphase and interphase fluorescent *in situ* hybridization (FISH) on primary tumor samples in conjunction with breakpoint-spanning YACs (2–5). These YACs contain several X chromosome-specific low copy repeat sequences, among which two ornithine amino-transferase-like pseudogene clusters, OATL1 and OATL2. Interestingly, we found that the occurrence of the two breakpoints correlates with the histologic phenotypes of the tumors, i.e., those with a breakpoint near the OATL1 region display a biphasic morphology, whereas most of the tumors with a breakpoint near OATL2 are monophasic (4,6,7). Recently, we isolated a chimaeric genomic (X;18) fragment containing the synovial sarcoma-specific breakpoint region (8). By using chromosome 18-specific single copy probes from this fragment, rearrangements were observed in tumors carrying translocation breakpoints in the vicinity of either OATL1 or OATL2, suggesting that a single gene on chromosome 18 is probably involved in both types of tumors (8). Subsequently, a chimaeric (X;18) cDNA clone was isolated by Clark *et al.* (9) and the contributing genes were referred to as SYT (chromosome 18) and SSX (X chromosome). Via RT-PCR, using SYT and SSX-specific primers, a 585 basepair (bp) chimaeric fragment could be amplified in several synovial sarcomas. No details were provided by these authors about the cytogenetic and/or histologic characteristics of the tumors studied.

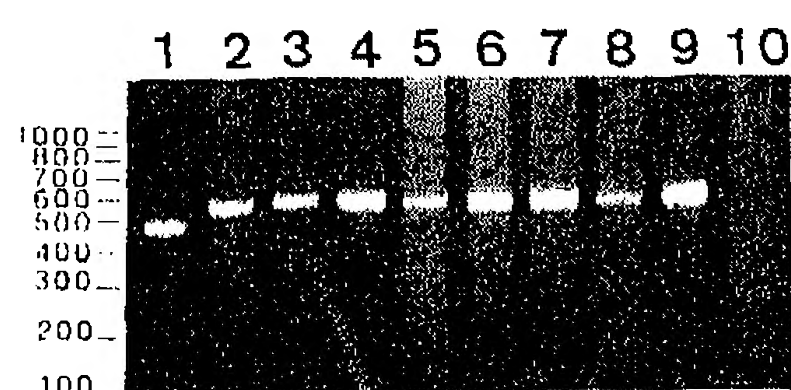

Figure 1. RT-PCR products (35 cycles; 96°C 1 min; 47°C 1 min; 72°C 3 min) of synovial sarcoma (1–9) and control renal tumor (10) samples, after agarose gel (2%) electrophoresis. As primers SYT: 5' CAACAGCAAGA-TGCATACCA 3' and SSX: 5' CACTTGCTATGCACCTGATG 3' were used (see Fig. 2). Synovial sarcomas: lanes 1 and 5–9: 28775/90, 2374/90, 23.303B, KN, 4873/92 and 20521/88 (see refs 4,7); lane 2: 293090 (unpublished case); lane 3: somatic cell hybrid H1synsarc (ref. 10); lane 4: a tumor-derived cell line (unpublished result). Fragment lengths are indicated in base pairs.

We have carried out RT-PCR on RNAs extracted from a series of nine independent synovial sarcomas, including one cell line and one tumor-derived somatic cell hybrid using the SYT/SSX primer set reported by Clark *et al.* (9). As a control a t(X;18)-negative renal cell carcinoma was included in our assays. All synovial sarcomas used in this series have been subjected to breakpoint analysis by FISH (4,7 and unpublished

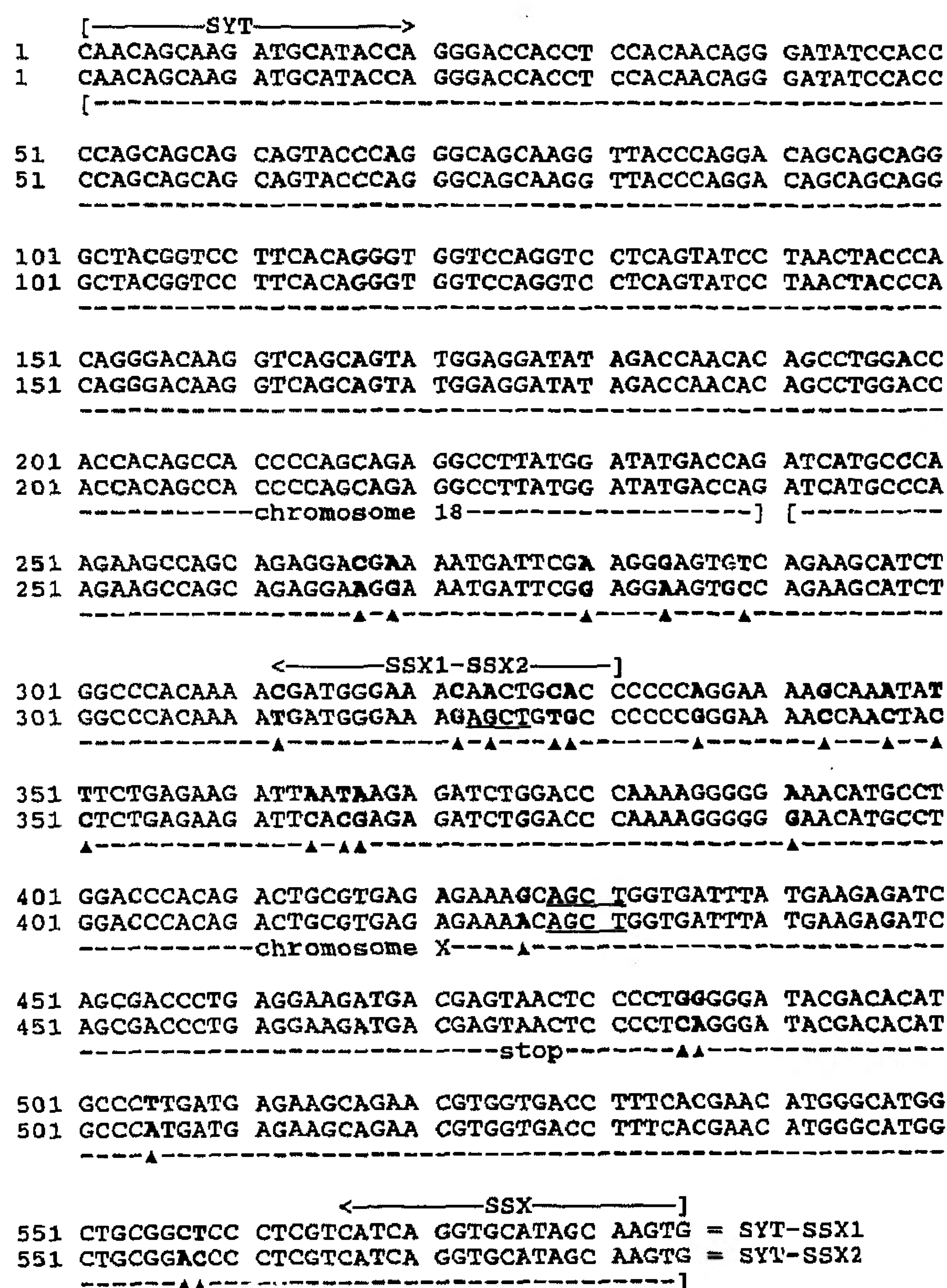

Figure 2. Comparison of the two different types of sequences found among the SYT/SSX PCR products displayed in Figure 1. Base differences are indicated by arrowheads. *Alu*I restriction sites are underlined. Locations of SYT, SSX and SSX1/2 specific primers are overlined (with directional arrows). The end of the open reading frame is indicated by 'stop'. The chromosomal regions of origin are marked between brackets.

*To whom correspondence should be addressed

Figure 3. *Alu*I restriction digests of SYT/SSX PCR products from synovial sarcomas. Lanes A,B: cases 4,5 and lanes C,D: cases 8,9 (see Fig. 1). Lanes E,F: undigested SYT/SSX PCR products of case 8 (585 bp) and case 1 (499 bp), respectively. Lane G: *Alu*I cleavage products of case 1. Fragment lengths are indicated in base pairs.

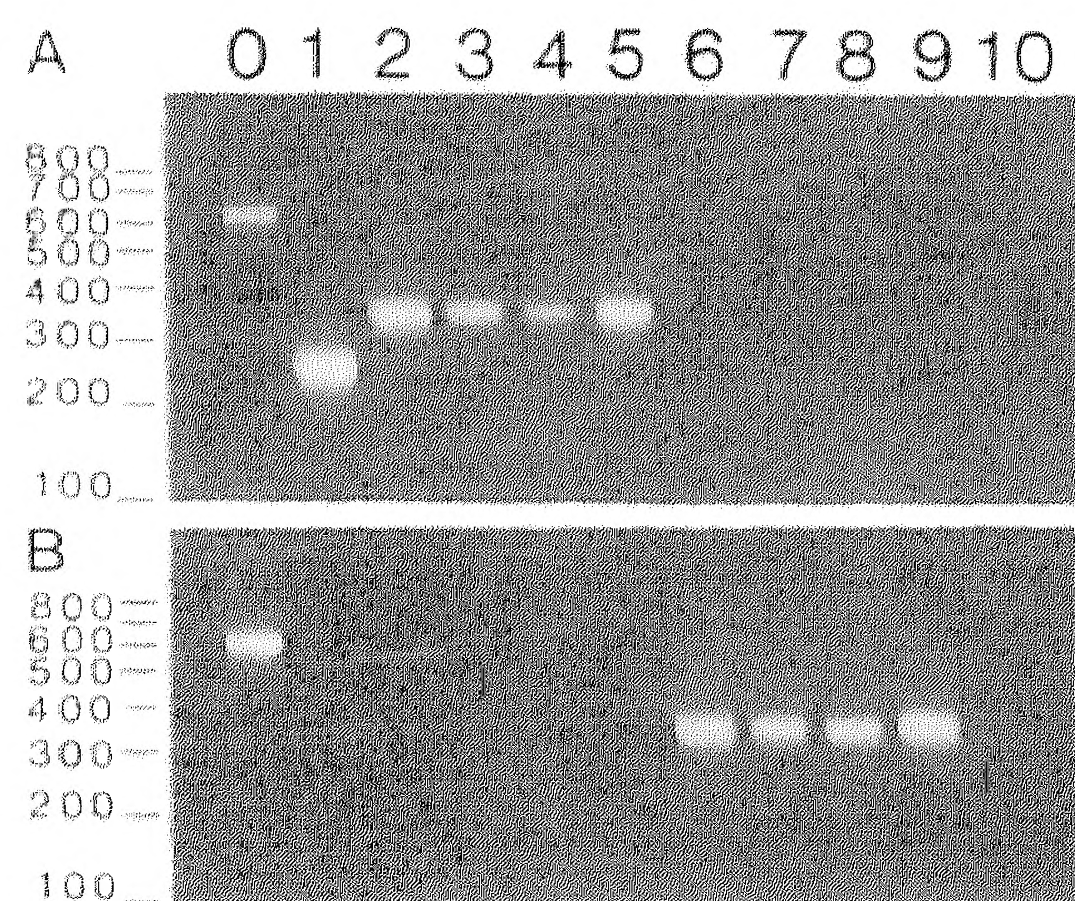

Figure 4. (**A**) 'Nested' PCR (30 cycles; 96°C 1 min; 48°C 1 min; 72°C 3 min) on SYT/SSX PCR products from cases 1–9 with the primers SYT (Figs 1,2) and SSX1: 5′ GGTGCAGTTGTTTCCCATCG 3′ (Fig. 2). (**B**) 'Nested' PCR as in A with primers SYT (Figs 1, 2) and SSX2: 5′ GGGACAGCTC-TTTCCCATCA 3′ (Fig. 2). Panels A and B: Lane 0, SYT/SSX PCR product of 585 bp as in Figure 1. Lane 10, control renal tumor. Fragment lengths are

were obtained from synovial sarcomas exhibiting either of the two alternative X-chromosomal breakpoints, we set out to clone and sequence the different amplified fragments. In all cases, sequences turned out to be identical for the region corresponding to chromosome 18 (SYT). However, consistent basepair changes were observed at 25 different positions (Fig. 2, arrowheads) in the X-chromosomal (SSX) segments of the PCR products derived from tumors carrying breakpoints near either OATL1 (1–5) or OATL2 (6–9). These basepair changes include an *Alu*I restriction site (underlined in Figure 2). Correspondingly, *Alu*I cleavage of the different PCR fragments resulted in 429 and 156 bp fragments (Fig. 3, lanes A and B) in the first group of tumors, and 324, 156 and 105 bp fragments (Fig. 3, lanes C and D) in the second group, respectively. Again, the 499 bp PCR product from case 1 yielded different results (Fig. 3, lanes F and G).

Specific primers (referred to as SSX1 and SSX2) were developed corresponding to a region exhibiting five bp differences (overlined in Figure 2). Subsequent RT-PCR analysis revealed the specific amplification of fragments of expected size (331 bp) in tumors 2–5 with SYT-SSX1, and in tumors 6–9 with SYT-SSX2 primer sets, respectively (Fig. 4). Again, tumor 1 gave an aberrantly sized fragment with only the SYT-SSX1 primer set. This indicates that the SYT-SSX1 and SYT-SSX2 primer sets are also informative in case of aberrant SYT-SSX fusion products.

From these results we conclude that indeed two related but distinct X-chromosomal genes (SSX1 and SSX2; located near OATL1 and OATL2 in Xp11.2, respectively) are rearranged in different subsets of t(X;18)(p11.2;q11.2)-positive synovial

indicated in base pairs.

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1..Ile Met Pro Lys Lys Pro Ala Glu Asp Glu Asn Asp Ser Lys Gly
1..Ile Met Pro Lys Lys Pro Ala Glu Glu Gly Asn Asp Ser Glu Glu
                                    ▲   ▲               ▲   ▲

16 Val Ser Glu Ala Ser Gly Pro Gln Asn Asp Gly Lys Gln Leu His
16 Val Pro Glu Ala Ser Gly Pro Gln Asn Asp Gly Lys Glu Leu Cys
        ▲                                           ▲       ▲

31 Pro Pro Gly Lys Ala Asn Ile Ser Glu Lys Ile Asn Lys Arg Ser
31 Pro Pro Gly Lys Pro Thr Thr Ser Glu Lys Ile His Glu Arg Ser
                    ▲   ▲   ▲                   ▲   ▲

46 Gly Pro Lys Arg Gly Lys His Ala Trp Thr His Arg Leu Arg Glu
46 Gly Pro Lys Arg Gly Glu His Ala Trp Thr His Arg Leu Arg Glu
                        ▲

61 Arg Lys Gln Leu Val Ile Tyr Glu Glu Ile Ser Asp Pro Glu Glu
61 Arg Lys Gln Leu Val Ile Tyr Glu Glu Ile Ser Asp Pro Glu Glu

76 Asp Asp Glu stop    SSX1 end
76 Asp Asp Glu stop    SSX2 end
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Figure 5. Amino acid comparison of the X chromosomal part of the fusion proteins corresponding to the two different sequences from Figure 2. Differences are indicated by arrowheads.

results). Tumors 1–5 were found to contain breakpoints near OATL1, and tumors 6–9 near OATL2. RT-PCR analysis of the nine sarcomas (Fig. 1, 1–9) resulted in fragments of expected size (585 bp) in all cases except one, in which a shorter product (Fig. 1, lane 1; 499 bp, details to be described elsewhere) was observed. The control renal tumor sample (Fig. 1, lane 10) was negative, as expected. Since the PCR products

sarcomas. In addition, we confirm our previous notion that the same gene on chromosome 18 (SYT) is involved in cases exhibiting either of the two alternative X-chromosomal breakpoints. The two X-chromosomal genes SSX1 and SSX2 differ at 25 positions within the 585 bp SYT-SSX RT-PCR product (93% homology). These differences result in 13 amino acid changes (Fig. 5, arrowheads). As most of these changes are non-conservative, they are expected to have effects on protein folding and function. Further analysis at the protein level is required to resolve the question as to how such changes are related to the ultimate tumor phenotype (monophasic versus biphasic). In this context, the exceptional case 1 may be particularly informative.

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