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CLUSTERING OF DELETIONS ON CHROMOSOME 13 IN BENIGN AND LOW-MALIGNANT LIPOMATOUS TUMORS

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Deletions and structural rearrangements of the long arm of chromosome 13 are frequently observed in benign and low-malignant lipomatous tumors, but nothing is known about their molecular genetic consequences. We assessed the karyotypes of 40 new and 22 previously published cases (35 ordinary lipomas, 15 spindle cell/pleomorphic lipomas, 2 myxolipomas, 1 angiomyxolipoma and 9 atypical lipomatous tumors) with chromosome 13-abnormalities, and found bands 13q12–22 to be frequently affected. Twenty-seven cases with structural abnormalities within this region were selected for breakpoint and deletion mapping by metaphase fluorescence *in situ* hybridization (FISH), using a set of 20 probes. Deletions were found in 23 of 27 cases. The remaining 4 cases had seemingly balanced rearrangements. The breakpoints were scattered but clustered to band 13q14, and in all cases with unbalanced abnormalities, a limited region within band 13q14 was partially or completely deleted. A deletion within band 13q14 was found together with a breakpoint on the other homologue in 5 cases, 4 of which could be tested further with regard to the status of the retinoblastoma (*RB1*)-gene. In all 4 cases, only 1 copy of the gene was deleted. In addition to the breaks and deletions in the vicinity of the *RB1*-locus, several other regions of 13q were recurrently affected, e.g., in the vicinity of the hereditary breast cancer (*BRCA2*; 13q12)- and lipoma HMGIC fusion partner (*LHFP*; 13q13)- genes. Our findings strongly indicate that deletion of a limited region (~2.5 Mbp) within 13q14, distal to the *RB1*-locus, is of importance in the development of a subset of lipomatous tumors.
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Key words: *lipoma; chromosome 13; deletion; fluorescence* in situ *hybridization; cytogenetics*

Adipocytic tumors constitute the largest subgroup of soft tissue tumors. Several histopathologic subtypes, associated with different clinical features, have been recognized.[1] Cytogenetic analysis of adipose tissue tumors has shown that the various histopathologic subtypes are characterized by distinctive clonal chromosomal abnormalities.[1,2] Ordinary lipomas typically harbor abnormalities involving 12q13–15, 6p or 13q,[3,4] hibernomas exhibit 11q13 abnormalities,[5] lipoblastomas are characterized by 8q11–13 abnormalities[6] and spindle cell and pleomorphic lipomas typically display complete or partial loss of 13q and chromosome 16.[7] Among the malignant adipose tissue tumors, the presence of giant chromosome markers and ring chromosomes, most often containing amplified chromosome 12-material, signifies atypical lipomatous tumors,[8] and the translocation t(12;16)(q13;p11) is pathognomonic for myxoid/round cell liposarcoma.[9] For most of these abnormalities, the molecular genetic consequences have been disclosed, revealing that the cytogenetic aberrations result in fusion or amplification of specific genes.

One of the more frequent abnormalities among benign and low-malignant adipose tissue tumors, *i.e.*, rearrangement of 13q, has not yet been investigated at the molecular level. A survey of all published karyotypes from lipomatous tumors[2] revealed that rearrangement of, or monosomy for, chromosome 13 had been detected in 72 of 463 cases (16%). Among the different histotypes, the frequency of chromosome 13-abnormalities varies greatly, ranging from 0% in lipoblastoma and hibernoma to 67% in spindle cell and pleomorphic lipomas (Table I). An assessment of the breakpoints involved in structural rearrangements shows 3 bands to be more frequently affected, *i.e.*, 13q12, 13q14 and 13q22, and the region spanning 13q12–22 to be most frequently deleted (Fig. 1). The aim of the present study was to delineate, by fluorescence *in situ* hybridization (FISH), the localization of breakpoints and the extent of deletions on chromosome 13 among benign and low-grade malignant lipomatous tumors.

MATERIAL AND METHODS

Patients

The material consisted of 62 benign and low-malignant lipomatous tumors that had been cytogenetically analyzed at the Department of Clinical Genetics in Lund, Sweden, at the Center for Human Genetics in Leuven, Belgium or at the Laboratoire de Génétique in Nice, France. All cases included in the study were selected on the basis of their showing clonal loss and/or structural rearrangement of chromosome 13. Of the 62 tumors, 53 were diagnosed as benign lipomas (35 ordinary lipomas, 15 spindle cell/pleomorphic lipomas, 2 myxolipomas and 1 angiomyxolipoma) and 9 as atypical lipomatous tumors. Four of the samples were from local recurrences, whereas the remaining 58 were from primary tumors.

Cell culture and cytogenetic analysis

Short-term culturing, harvesting and cytogenetic analysis were performed according to standard methods[10] and the karyotypes were written according to ISCN.[11] Twenty-two of the karyotypes have been published before.[4,7,12–17]

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Probes for FISH

Yeast artificial chromosome (YAC) probes were selected from the Whitehead Institute for Biomedical Research, Cambridge (http://www-genome.wi.mit.edu/; October 2000) and provided by le Centre d′Etudes du Polymorphisme Humain (CEPH), (http://www.cephb.fr/infoclone.html), Paris. Bacterial artificial chromosome (BAC) probes were selected from the human RP11-BAC library based on the status presented by NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search; October 2001) and provided by the BAC-PAC resources at the Roswell Park Institute, New York (http://genomics.roswellpark.org/human/overview.html). The choice of probes was based on the findings from the preceding cytogenetic analysis of the samples and the relative positions of known sequence tagged sites (STS). The positions of the YAC and BAC clones in relation to each other and to known genes in 13q12–21 are presented in Figure 2. Whole-chromosome painting probes (AppligeneOncor, Illrich, France and Vysis, Downers Grove, USA) were used to distinguish unequivocally chromosome 13-material and other chromosomes when necessary.

BAC DNA was extracted using standard protocols. Total yeast DNA was extracted from YAC yeast clones, and human sequences were specifically amplified by using the primers ILA3′ and ILA5′, as described by Höglund *et al.*[18] The PCR-amplified YAC DNA and the BAC DNA were subsequently labeled by random hexamer priming (Megaprime DNA labeling system, Amersham, Buckinghamshire, UK), either indirectly with biotin-16-deoxyuridine-triphosphate (dUTP) (Roche, Mannheim, Germany), digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) or estradiol-15-dUTP (Roche), or else directly with Cy3-conjugated deoxycytosinetriphosphate (dCTP) (Amersham). The labeled DNA was passed through a sepharose column and 2 μg of labeled probe were precipitated with carrier DNA (12 μg Human Cot DNA, 56 μg salmon sperm DNA), 0.5 of the volume ammonium acetate (7.5 M) and 2.5 volumes absolute ethanol before being dissolved in 50% formamide/2× saline sodium citrate (SSC) hybridization solution to a final concentration of 20 ng/μl. The probes were denatured at 72°C and then prehybridized at 37°C for 45 min. The probe combinations were made immediately before application to the slides.

TABLE I – THE FREQUENCY OF CHROMOSOME 13 ABERRATIONS AMONG PREVIOUSLY PUBLISHED LIPOMATOUS TUMORS[1]

Lipomatous tumor	Number of cases	Number of cases with chromosome 13 aberrations[2] (%)
Lipoma[3]	229	41 (18)
Lipoblastoma	16	0 (0)
Hibernoma	7	0 (0)
Spindle cell- and pleomorphic lipoma	9	6 (67)
Atypical lipomatous tumors[4]	98	12 (12)
Myxoid/round-cell liposarcoma[5]	87	4 (5)
Pleomorphic- and dedifferentiated liposarcoma	17	9 (53)

[1]Mitelman Database of Chromosome Aberrations in Cancer (2002).–[2]Including loss and structural rearrangement.–[3]Including ordinary lipoma, angiomyolipoma, parosteal lipoma, fibrolipoma, angiolipoma, chondroid lipoma and myxolipoma.–[4]Including atypical lipoma and well-differentiated liposarcoma.–[5]Including mixed liposarcoma.

FISH

The metaphase spreads were aged over-night at 60°C, washed in 2× SSC for 2 hr at 60°C and finally air-dried. The preparations were washed in 1× phosphate-buffered saline (PBS) for 5 min and then enzymatically digested with pepsin (10 mg/ml in 0.01 M HCl), followed by a wash in 1× PBS for 5 min. The slides were postfixed in 1% formaldehyde for 5–10 min, washed once in 1× PBS for 5 min and finally dehydrated in an ethanol series (70%, 85% and 100%). Denaturation of the chromosomes was done in 70% formamide/2× SSC for 40 sec to 2.5 min at 74°C, followed by dehydration in an ethanol series. Labeled and denatured probes (100 ng/probe) were added to each slide and the spread was covered with an 18 × 24-mm coverslip and sealed with rubber glue. Alternatively, the chromosomes were denaturated by adding the labeled and denaturated probe solution (see above) directly to the slide, which was then sealed with coverslip and rubber glue and finally heated at 74°C for 3–4 min. Hybridization was done over-night in a humid chamber at 37°C. Posthybridization washes were done in 0.4 × SSC for 2 min at 70°C, followed by a 3 min rinse in 4T (4 × SSC, 0.05% TWEEN-20, Sigma Chemical Co., St. Louis, MO) for ordinary preparations, and TNT (0.1 M Tris-HCl pH=7.5, 0.15 M NaCl, 0.05% TWEEN-20, Sigma Chemical Co.) for *in situ* preparations.

Unspecific binding of antibodies was prevented by treatment with a blocking solution [4 M (5% fat-free dry milk in 4 × SSC) for ordinary preparations, TNB (Boehringer Mannheim) for *in situ* preparations] for 15–30 min at room temperature, followed by a rinse in 4T/TNT for 3 min. Biotin-labeled probes were detected with avidin-conjugated Cy3, Cy5 (Amersham) or fluorescein-isothiocyanate (FITC) (Sigma Chemical Co.). Digoxigenin-labeled probes were detected with sheep-anti-digoxigenin antibodies conjugated to FITC (Sigma Chemical Co.). The slides were treated

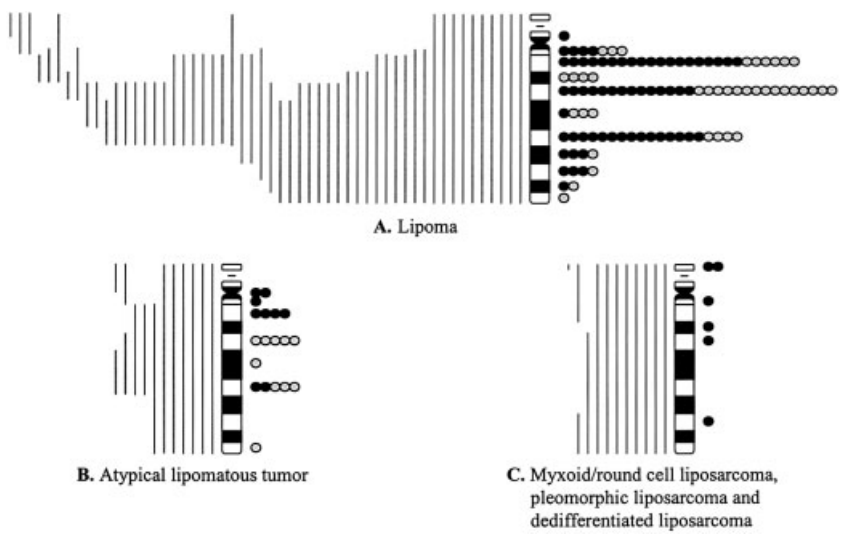

FIGURE 1 – Ideograms of chromosome 13 that show the distribution of breakpoints and deletions in different subtypes of lipomatous tumors as detected by chromosome banding analysis. Solid line and closed circle: deletions and breakpoints as reported in published karyotypes.[2] Shaded line and shaded circle: deletions and breakpoints of cases included in the present study. Spindle cell/pleomorphic lipomas are included among lipomas.

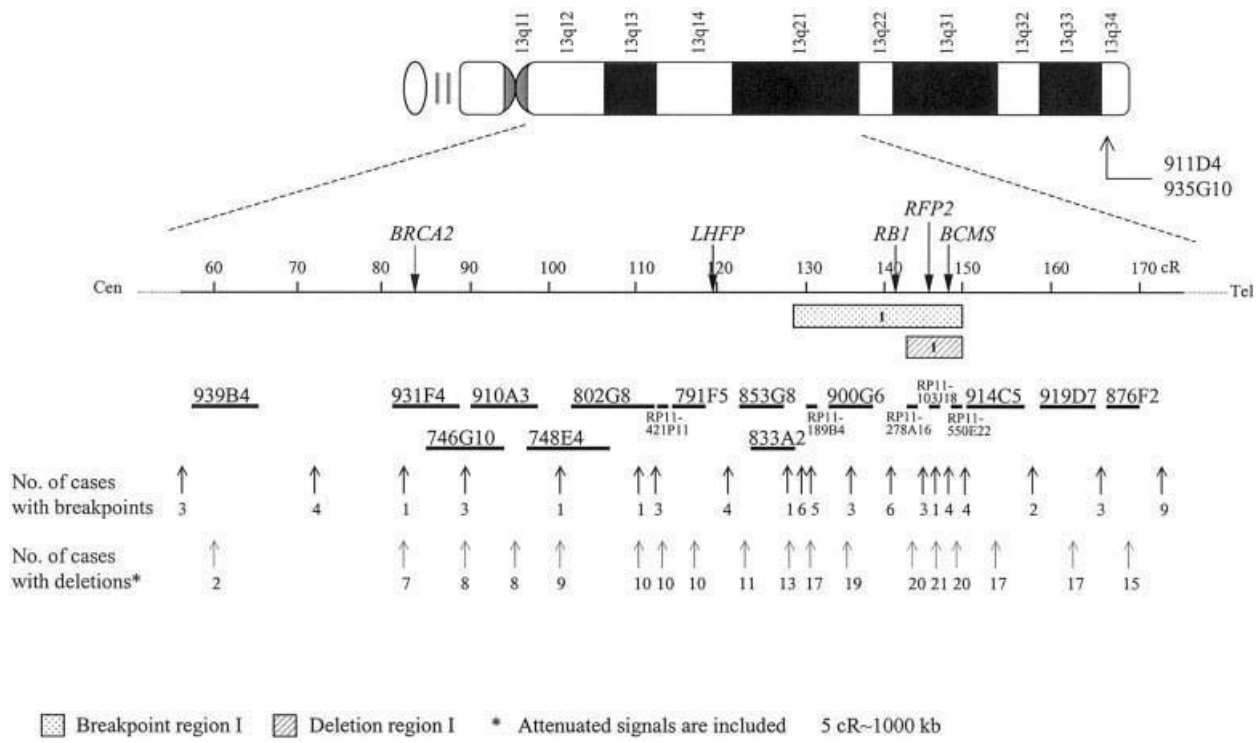

FIGURE 2 – Physical map of central 13q according to data available from the Whitehead Institute. The approximate sizes of the YAC and BAC clones are relative to the included cR-bar.

with the appropriate mixture of antibodies for 30 min at room temperature, followed by 3× 5 min rinses in 4T/TNT and a final dehydration in an ethanol series. For the detection of estradiol-labeled probes, the slides were first treated with rabbit-anti-estradiol immunoglobulin G (IgG) (Roche) for 30 min, followed by a rinse in 4T for 3 min. In a second step, the slides were treated for 30 min with goat-anti-rabbit IgG coupled to Cy5 (Amersham), in combination with additional antibodies needed for the detection of other probes. After a final 3 × 5 min rinsing in 4T, the slides were dehydrated in an ethanol series. Slides hybridized only with directly labeled probes were dehydrated in an ethanol series immediately after the posthybridization wash. For detection of nuclei and evaluation of probes, the spreads were counterstained with 0.5 mg/ml 4,6,diamidino-2-phenylindole (DAPI, Boehringer Mannheim) and mounted in a 2% 1,4-diazabicyclo-[2,2,2]-octane (DABCO, Sigma Chemical Co.) solution. The slides were examined in an Axioskop microscope (Zeiss) coupled to a CytoVision Ultra system (Applied Imaging), with a cooled charge-coupled device camera. A minimum of 10, typically 20–30, well-spread metaphase cells were evaluated on each slide.

RESULTS

Cytogenetics

All 62 cases included in the present study had abnormal karytopes with loss and/or structural rearrangement of chromosome 13. In Table II, the complete karyotypes of 40 new cases as well as 7 of the 22 previously published cases[4,7,12–17] that were used for FISH analysis are presented. The structural changes could be dichotomized into balanced and unbalanced rearrangements. In 14 cases, a seemingly balanced exchange was disclosed, and in 5 of them the exchange was present as the sole abnormality. In 1 other tumor,[12] a clone with a balanced t(13;17) was found together with a clone with del(13). Unbalanced structural rearrangements were due to translocations (11 cases) and/or interstitial (11 cases) or terminal (12 cases) deletions. In 1 other tumor (case 35), the rearrangement resulted in loss of 13q12–13 as well as gain of 13q14-qter. In 9 of the cases, the unbalanced structural rearrangement of chromosome 13 was the only change seen. In 12 cases, the entire chromosome 13 was lost, always together with other numerical or structural abnormalities.

In 28 cases, the structural abnormalities of chromosome 13 were accompanied by another abnormality typically associated with lipomatous tumors, *i. e.*, rearrangement of 12q13–15 (7 cases) or 6p21-pter (6 cases), loss of chromosome 16 material (9 cases) or the presence of ring chromosomes (6 cases).

FISH

Material for metaphase FISH was available from 27 of the 50 cases with structural chromosome 13-rearrangements (Table II). Among these 27 cases, the karyotypes indicated that all bands on chromosome arm 13q were involved at least once, with a clustering of breaks to 13q12–q22 (Fig. 1). Hence, a series of FISH-probes for this region was used to delineate the distribution of breakpoints (Fig. 2).

Breakpoints and deletions. In 4 tumors (cases 4, 5, 7 and 28 clone B), FISH analysis could not reveal any gain or loss of chromosome 13-material, which was in agreement with the karyotypic data. Three of them had balanced translocations (cases 4, 5 and 28 clone B) and 1 had an insertion (case 7). In 2 of the tumors, (cases 5 and 28 clone B), the breakpoints were found in the same region (Fig. 3).

In the other 23 cases, unbalanced rearrangements with loss of 13q-material were detected by FISH analysis (Fig. 3). These included all cases that at chromosome banding analysis were interpreted to have deletions or unbalanced translocations, as well as 5 of the 9 cases that were interpreted to have balanced rearrangements. In 7 cases, terminal deletions were seen and in 17 cases the structural changes resulted in interstitial loss of material. The breakpoints were widely distributed, and in 18 cases, more than 1 break was seen (Fig. 2). Nine cases had

TABLE II – CLINICAL AND CYTOGENETIC DATA ON 47 BENIGN AND LOW-MALIGNANT LIPOMATOUS TUMORS WITH ABERRATIONS OF CHROMOSOME 13

Case	Sex/age	Location/depth[1]	Size[2]	Karyotype[3]
Lipoma				
1	F/51	Buttock/S	10	46,XX,**t(12;13)(q22;q14)**[42]
2[6]	M/62	Upper arm/D	7	46,XY,**t(13;13)(q13;q33)**[19]
3[6]	M/35	Thigh/D	3	46,XY,**t(13;21)(q22;q22)**[3]
4[4,6]	M/59	Upper arm/D	10	46,XY,t(4;5)(p16;q13),**t(12;13)(p11;q14)**[18]/92–94,idemx2[2]
5[6]	M/55	Shoulder/S	8	46,XY,**t(4;13)(q?21;q?32)**,?inv(12)(q14q21)[4]/46,XY,del(6)(q23),?inv(12)(q14q21)[6]
6	F/66	Thigh/D	23	46,XX,**?inv(13)(q12q14)**,?add(22)(q12)[22]
7[6]	F/42	Upper arm/S	6	46,XX,t(4;15)(p15;q15),t(5;9)(q22;q32),**ins(8;13)(q24;q34q14)**,add(16)(q13),der(20)t(16;20)(q13;q12)[25]
8[6]	M/43	No data	–	46,XY,r(12)(p13q1?5),**der(13)t(12;13)(q15;q11)**[20]
9[5,6]	M/63	Shoulder/D	13	46,XY,**der(12)t(12;13)(q15;q22),der(13)t(12;13)(q15;q14)**[25]
10[4,6]	M/49	No data	–	46,XY,**der(6)t(6;13)(p22;q22),del(13)(q14)**[22]
11	F/69	Face/S	3	45,XX,**der(2)t(2;13)(q21;q14),−13**[10]
12[6]	M/52	Shoulder/S	–	44,XY,del(2)(q34q36),**−13,−13,der(16)t(13;16)(q12;q12)**[12]
13	F/81	Thigh/D	5	46,XX,−11,−12,**der(13)t(12;13)(q22;q13)**,+der(?)t(?;11)(?;q13),+?r[8]
14	M/38	Thigh/S	12	46,XY,**der(1)t(1;13)(q21;q14)**,der(8)t(1;8)(q21;p21),der(12)t(8;12)(p21;p13),**add(13)(q11)**[22]
15	M/63	Lower arm/S	3	46,XY,del(1)(p13),der(2)t(1;2)(p32;q3?7),?del(4)(q31),−5,add(6)(p23),?add(8)(q1?),+9,add(10)(q24),der(10)t(5;10)(q15;q24),add(11)(p11),**?der(13)t(8;13)(q13;q14)**,add(21)(q22)[23]
16	M/47	Thigh/D	9	46,XY,**del(13)(q12q22)**[10]
17[6]	M/56	Neck/S	5	46,XY,**del(13)(q13q14)**[13]
18[4,6]	F/45	Upper arm/D	5	48,XX,+8,+12,**del(13)(q11q14)**[25]
19[6]	M/35	Breast/S	5	46,XY,**del(13)(q14)**[20]
20[6]	F/47	Neck/S	4	46,XX,t(2;18)(q33;p11),**del(13)(q21)**[14]
21	M/66	Back/S	3	46,XY,t(3;6)(p25;q13),add(4)(p16),**?del(13)(q11)**[cp3]
22	F/44	Shoulder/S	9	45,XX,del(6)(q21),**del(13)(q14)**,−16[22]
23[6]	M/47	Back/S	10	45,XY,der(3)t(3;14)(q24;q24),del(6)(p22),inv(9)(p13q13),**del(13)(q14)**,−14[10]
24	M/55	Breast/D	16	45,XY,del(6)(q13q25),−10,**add(13)(q21)**[10]
25	F/45	Shoulder/D	10	46,XX,add(6)(p2?2),**?add(13)(q12)**,−18,add(18)(q12),+mar[24]
26	F/72	Shoulder/S	9	46,XX,der(3)add(3)(p25)del(3)(q21),del(12)(q13),**add(13)(q12)**,add(16)(p11)[20]
27[5]	F/63	Shoulder/S	4	45,XX,t(1;6)(p21;q27),add(3)(q27),**−13**,del(17)(p11)[cp10]
Angiomyxolipoma				
28[4,6]	F/60	Thigh/S	6	46,XX,**der(7)t(7;13)(p13;q21)**,t(8;12)(q13;p13),**del(13)(q21)**[6]
Myxolipoma				
29[6]	M/51	Shoulder/D	11	45,XY,t(3;12)(q27–28;q15),t(7;17)(q11;q24),**−13,der(16)t(13;16)(q31;q24)**[15]
30	M/59	Trunk wall/S	5	58,XXY,+X,−1,−2,−3,−6,−9,−10,−11,**−13,−13**,−14,−15,−16,+19,−22[25]
Spindle cell lipoma				
31[6]	M/61	Neck/S	4	42,X,−Y,**t(1;13)(p36;q14)**,der(4;10)(q10;q10),−14,−16[9]
32[6]	M/66	Shoulder/S	15	46,XY,**del(13)(q21q22)**[16]
33[6]	M/63	Neck/D	9	46,XY,der(6)t(6;?19)(p22;?q11),**del(13)(q14q22)**[20]
34[6]	M/70	Face/S	1	46,XY,t(12;15)(q15;q25∼26),**del(13)(q14q33)**[10]
35	M/53	Shoulder/S	4	46,XY,t(4;6)(q25;p23),**der(11)t(11;13)(p15;q14),del(13)(q12q13)**[5]/46,idem,tas(Y;21)(p11;p13)[3]
36[4,6]	M/52	Neck/D	3	46,XY,t(2;16)(p16–21;p21),**del(13)(q12)**[12]
37[6]	F/53	Lower leg/D	4	46,XX,t(1;6)(p31;p12),**del(13)(q14)**[24]
38[4,6]	M/64	Neck/S	4	45,XY,der(5)t(5;6)(q11;p11),−6,add(10)(p11),**del(13)(q12)**[20]
39	M/69	Neck/S	5	44,XY,der(9)t(9;17)(p11;q11),**−13**,del(16)(q13),−17[10]
40	M/33	Neck/S	2	42–44,XY,add(3)(p13),add(6)(q15),add(8)(p11),**−13**,−14,der(15)t(14;15)(q11;q24),−16,del(17)(p11),add(21)(q11)[cp6]
41	M/78	Neck/S	–	58,XXY,+Y,−1,−2,−4,−6,−7,+8,−9,−10,−12,**−13**,−14,−16,−17,−22[15]
Atypical lipomatous tumor				
42[6]	M/47	Trunk wall/S	4	46,XY,**t(13;13)(q14;q34)**[25]
43[6]	M/63	Neck/S	11	46,Y,**t(X;13)(q28;q14)**[20]
44	F/64	Thigh/D	2	46,XX,**t(12;13)(p13;q22)**[10]/47,idem,+r[14]/47,idem,+mar[2]
45[6]	M/52	Back/S	16	46,XY,r(12),**del(13)(q21q22),ins(13;17)(q14;q12q24)**[10]/92,idemx2[5]/polyploid,complex[5]
46	M/58	Lower leg/D	9	45,XY,der(12)add(12)(p11)add(12)(q13),**?del(13)(q14),der(13)t(12;13)(q15;q14)**,−21[3]
47[6]	M/63	Back/S	2	46,XY,**del(13)(q14q22)**[28]

[1]S, superficial; D, deep-seated.–[2]Largest diameter in cm; –, unknown.–[3]Rearrangements involving chromosome 13 are high-lighted in bold characters.–[4]Previously published karyotypes.[4,13,16,17]–[5]Recurrence. All other karyotypes are from primary tumors.–[6]Further characterized by FISH in the present study.

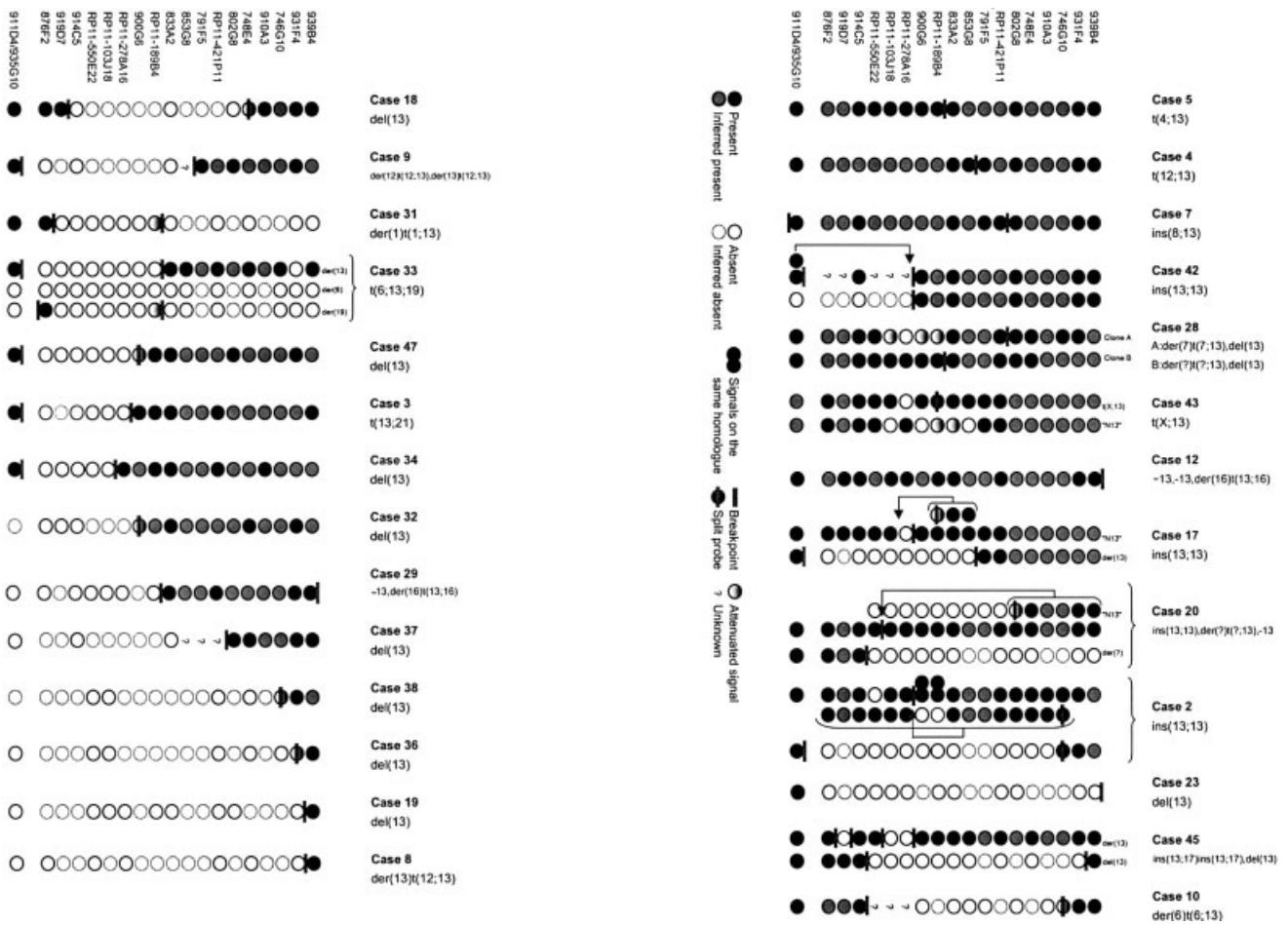

FIGURE 3 – Summary of results from the detailed FISH-mapping in 27 cases of lipomatous tumors. The case numbers and corresponding chromosome 13-changes are indicated at the top; the probes used are indicated to the left. The partial karyotypes refer to the FISH-findings.

breaks in the large region flanked by 876F2 and 911D4/935G10, 13 cases had breaks in the region covered by RP11-189B4 to RP11-550E22 (hereafter referred to as breakpoint region I), 6 cases had breaks between 833A2 and RP11-189B4 and 5 cases had breaks in the region covered by probes 931F4 and 746G10. Deletions varied considerably in size, ranging from loss of all analyzed sequences in the 13q12–21 region to loss of a single locus. The frequencies with which deletions were detected increased from the centromeric side towards the telomere, until a maximum (21 cases) was reached for probe RP11-103J18. The sequences recognized by probes RP11-278A16, RP11-103J18 and RP11-550E22 (hereafter referred to as deletion region I) were deleted in at least 20 cases. In 5 of 6 cases with a breakpoint between 900G6 and RP11-278A16, the corresponding region on the other chromosome 13 was deleted. To evaluate the status of the *RB1*-gene in these cases, a probe for the *RB1*-locus (RP11-305D15) was used in cases 2, 17, 42 and 43 (no material was available for case 45). In all of them, 1 copy of the genes was retained (data not shown).

Complex structural rearrangements. FISH analysis revealed complex structural rearrangements in 6 of the cases. In cases 2 and 42, the rearrangement appeared to be an ins(13;13) rather than a t(13;13). In case 42, the inverted ins(13;13) was accompanied by loss of material from 1 of the 2 homologues (Fig. 4*a*). In case 2, the exact nature of the multiple rearrangements of the 2 chromosomes 13 could not be elucidated in spite of extensive analysis. Most likely, at least 7 breakpoints were involved in insertions and interstitial deletions, resulting in net loss of material from both homologues (Fig. 4*b*).

Case 45 was at cytogenetic analysis thought to harbor an ins(13;17), with an interstitial deletion within the other chromosome 13-homologue. The FISH analysis showed that the inserted chromosome 17 segment contained an inserted fragment from chromosome 13. On the derivative chromosome 13, 2 regions in the vicinity of the inserted chromosome 17 segments were deleted (Fig. 4*c*). The other chromosome 13 homologue had an interstitial deletion.

Cases 33 and 17 were from the cytogenetic results expected to contain an interstitial deletion in combination with a normal homologue. In case 33, 1 homologue seemed normal but the other exhibited small interstitial deletions, but also a complex translocation that involved chromosomes 6 and 19 (Fig. 4*d*). In case 17, none of the 2 homologues was normal. The interstitial deletion observed at cytogenetic analysis was confirmed but part of the deleted segment was inserted into the "normal" homologue (Fig. 4*e*). Furthermore, the breakpoint on this second homologue was associated with a small deletion.

In case 20, which at cytogenetic analysis was interpreted to have a terminal deletion, an ins(13;13) as well as a translocation to an unknown chromosome was found. At least 3 breaks within 1 of the homologues led to deletion of 2 parts and translocation and insertion of the 2 other segments (Fig. 4*f*).

Histopathologic correlations. The 27 cases that could be analyzed by FISH included 14 ordinary lipomas, 7 spindle cell lipomas, 4

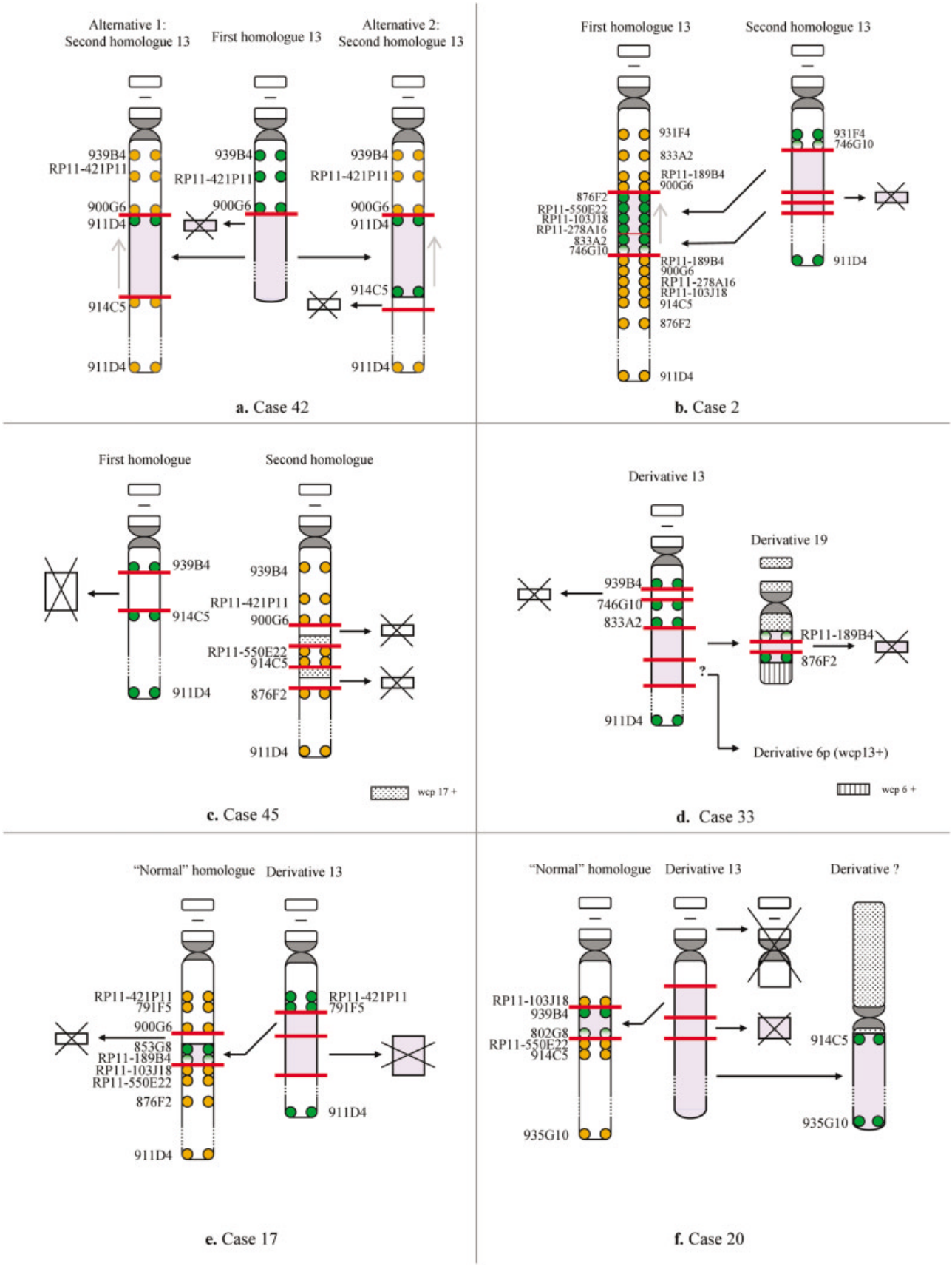

FIGURE 4 – (*a–f*) Clarification of FISH results in 6 tumors with complex rearrangements of chromosome 13. If not otherwise indicated, the corresponding homologue is normal with regard to the analyzed region. Translocated regions are illustrated as blue boxes and deleted regions as crossed boxes. Signals are illustrated as yellow or green circles to distinguish material from each of the 2 chromosome 13-homologues. Chromosomal breaks are highlighted as thick red lines. The orientation of the translocated segment is indicated by a gray arrow.

atypical lipomatous tumors, 1 myxolipoma and 1 angiomyxolipoma (Table II). The number of cases in each histopathologic subgroup being small, no attempt to correlate breakpoint distribution with morphology was made. However, it could be noted that all spindle cell lipomas carried rather simple chromosome 13-changes and that all of them displayed fairly large deletions of 13q, the minimal region of overlap being flanked by probes RP11-278A16 and 876F2 (Fig. 2). Furthermore, in 3 of 4 atypical lipomatous tumors, both homologues of chromosome 13 were involved in rearrangements. All 4 cases with balanced rearrangements were diagnosed as ordinary lipomas, only 1 of which possibly displayed another lipoma-associated aberration [?inv(12)(q14q21) in case 5].

DISCUSSION

Previous cytogenetic studies have shown that a substantial subset of adipose tissue tumors displays rearrangement or loss of chromosome 13.[1,2] In the present study, 62 cytogenetically analyzed cases of benign or low-malignant adipose tissue tumors, all harboring structural rearrangements and/or loss of chromosome 13, were collected (Table II and previously published karyotypes[7,12,14,15]). In approximately 1/4 of the cases, only 1 or no additional rearrangement could be detected, supporting the view that rearrangements of 13q constitute a separate, primary change in adipose tissue tumors. A survey of the karyotypic data on these and all previously published cases confirmed the nonrandom pattern of chromosome 13-rearrangements, with a clustering of breakpoints and deletions to the 13q12–q22 region (Fig. 1). We also assessed the correlation between karyotype and patient sex and age in 362 benign and low-malignant lipomatous tumors, including cases presented here and cases previously included in the Mitelman Database of Chromosome Aberrations in Cancer[2] (Table III). For ordinary lipomas, no difference was seen between cases with and without chromosome 13-rearrangements. For atypical lipomatous tumors, a tendency for cases with chromosome 13-rearrangements to be more common in men and at younger age was seen, but these associations did not reach statistical significance (Fisher's exact test).

A non-random distribution of 13q-rearrangements was confirmed by FISH analysis of 27 of the 62 cases and, not unexpectedly, several abnormalities undetected by cytogenetic G-banding analysis were disclosed by FISH analysis (Fig. 4). In 23 cases, FISH analysis revealed unbalanced rearrangements, including most of the cases where cytogenetic analysis had indicated balanced changes. The breakpoints were scattered and the extension of the deleted regions varied from the entire analyzed region to a more limited segment within 1 chromosome band. Despite the variable breakpoint distribution, 13 of the 23 cases displayed 1 or more breakpoints within breakpoint region I in chromosome band 13q14, with an ensuing partial or complete loss of this same region. The deletion breakpoint was within or closely proximal to RP11-189B4 in 15 cases, within the sequence recognized by 900G6 in 3 cases and between the sequences recognized by 900G6 and RP11-278A16 in 5 cases. The status of the *RB1*-locus, flanked by 900G6 and RP11-278A16, was thus analyzed further in the 4 cases in which a breakpoint between the sequences recognized by 900G6 and RP11-278A16 was found concomitantly with deletion of the corresponding region on the other homologue. However, in all these cases, only 1 copy of the gene was deleted, a finding that, of course, does not exclude functional inactivation of *RB1* through a point mutation or an aberrant methylation pattern, affecting the expression of the retained allele.[19]

Deletion of 13q is a recurrent karyotypic feature of several types of neoplasia, including solid tumors of epithelial, mesenchymal and neuronal origin as well as hematopoietic neoplasms.[2] Furthermore, deletions of 13q have been frequently detected by molecular genetic or molecular cytogenetic approaches in, *e.g.*, breast,[20] head and neck,[21] liver[22] and prostate cancer,[23] as well as in B-cell chronic lymphocytic leukemia[24] and multiple myeloma.[25] The molecular studies have revealed that loss of heterozygosity (LOH) is particularly common in 2 regions close to, but outside, the *RB1*-locus: 1 on the centromeric side and 1 on the telomeric side. The conclusion that the *RB1*-gene is not the main target for the deletions is supported by the lack of correlation between LOH at the *RB1*-locus and reduced *RB1*-expression in breast, prostate and head and neck tumors,[21,26,27] although contrasting reports have been reported for some breast[28] and liver cancers.[29] Interestingly, an association between a constitutional *RB1*-mutation and the development of lipoma has been suggested from an epidemiological study of 556 patients with hereditary retinoblastoma.[30] Although inactivation of the *RB1*-gene could not be excluded in the present study, the clustering of breaks and deletions opens the possibility for the presence and involvement of other target genes, which are located close to *RB1*. Deletion region I, which was lost in a minimum of 20 cases, harbors the genes *RFP2*/*LEU5* (Ret Finger Protein 2), *BCMS*/*LEU1* (B-Cell neoplasia associated gene with Multiple Splicing) and *BCMSUN*/*LEU2* (*BCMS*-Upstream Neighbour). These genes, localized 1–2 Mbp distal to *RB1*,[31] have all been implicated in B-cell chronic lymphocytic leukemia. So far, however, there are no convincing data that indicate that they are involved in the pathogenesis of leukemias,[32] let alone solid tumors.

In 5 cases with unbalanced rearrangements, deletion breakpoints were seen within, or closely distal to, 931F4 in chromosome band 13q12, and in 7 cases, this probe was deleted. Probe 931F4 contains the marker *D13S260*, which maps to the *BRCA2*-gene.[33] As with the *RB1*-locus, the involvement of the *BRCA2*-gene cannot be excluded unless molecular genetic studies are performed, but it could be noted that no increase in lipoma incidence among *BRCA2*-mutation carriers has been reported. Four tumors shared a breakpoint in the distal part of band 13q13. A possible target for these rearrangements is the Lipoma *HGMIC* Fusion Partner (*LHFP*)-gene, associated with the marker *WI-7467*,[34] which is located between probes 791F5 and 853G8 according to the Whitehead Institute. One of them displayed an exchange with band 12q15, but no material was available for FISH analysis of the *HMGA2* (a.k.a. *HMGIC*)-gene in this case.

The number of cases that could be subjected to FISH analysis was too small to identify any significant differences among the various histopathologic subsets of lipomatous tumors. However, with regard

TABLE III – CLINICAL FEATURES OF BENIGN AND LOW-MALIGNANT LIPOMATOUS TUMORS WITH AND WITHOUT 13q-ABNORMALITIES[1]

Tumor type[2]	Sex		Age (years)		
	Male (%)	Female (%)	<30 (%)	30–59 (%)	>59 (%)
L without 13q-abnormalities	94 (52)	86 (48)	4 (3)	86 (63)	46 (34)
L with 13q-abnormalities	33 (52)	30 (48)	–	34 (67)	17 (33)
SL/PL without 13q-abnormalities	1 (33)	2 (67)	–	2 (67)	1 (33)
SL/PL with 13q-abnormalities	15 (88)	2 (12)	–	7 (41)	10 (59)
ALT without 13q-abnormalities	42 (51)	40 (49)	–	23 (32)	50 (68)
ALT with 13q-abnormalities	12 (71)	5 (29)	–	6 (40)	9 (60)

[1]Based on previously published cases[2] (2002), and all cases included in the present study. The 13q-abnormalities include structural rearrangements of 13q as well as loss of the entire chromosome 13. Information on patient age was not available for all cases.–[2]L, ordinary lipoma; SL/PL, spindle cell/pleomorphic lipoma; ALT, atypical lipomatous tumor (atypical lipoma and well-differentiated liposarcoma).

to 13q-abnormalities, it seems as if homozygous deletions that involve band 13q14 may be more common in ALTs, that balanced translocations are characteristic for benign lipomas and that spindle cell lipomas display relatively large 13q-deletions (Fig. 2). Thus, it cannot be excluded that subtype-specific genetic mechanisms are operative, in spite of the fact that they all exhibit a clustering of abnormalities to the same limited chromosome segment.

Taken together, the results of this FISH study, which is the first attempt to delineate chromosome 13-rearrangements in adipose tissue tumors, show that chromosome 13 is involved in a variety of rearrangements that are likely to have many different consequences at the DNA level. Most importantly, however, the results strongly indicate that deletions that cover a limited segment (~2.5 Mbp) of chromosome band 13q14, distal to the *RB1*-gene, are of importance in the development of a subset of adipose tissue tumors. Whether these deletions point to the existence of 1 or more tumor suppressor genes of importance for adipose tissue tumorigenesis will have to be investigated by molecular genetic techniques. Bearing in mind the rapid progress in the mapping of the human genome, it should soon be possible to analyze the expression status of all genes within the critical region, which might be a necessary approach in order to unravel haplo-insufficiency as a pathogenetic mechanism.

REFERENCES

1. Fletcher CDM, Åkerman M, Dal Cin P, de Wever I, Mandahl N, Mertens F, Mitelman F, Rosai J, Rydholm A, Sciot R, Tallini G, van den Berghe H, et al. Correlation between clinicopathological features and karyotype in lipomatous tumors: a report of 178 cases from the chromosomes and morphology (CHAMP) collaborative study group. Am J Pathol 1996;148:623–30.
2. Mitelman Database of Chromosome Aberrations in Cancer. Mitelman F, Johansson B, Mertens F, eds. http://cgap.nci.nih.gov/Chromosomes/Mitelman.
3. Sreekantaiah C, Leong SPL, Karakousis CP, McGee DL, Rappaport WD, Villar HV, Neal D, Fleming S, Wankel A, Herrington PN, Carmona R, Sandberg AA. Cytogenetic profile of 109 lipomas. Cancer Res 1991;51:422–33.
4. Mandahl N, Höglund M, Mertens F, Rydholm A, Willén H, Brosjö O, Mitelman F. Cytogenetic aberrations in 188 benign and borderline adipose tissue tumors. Genes Chromosomes Cancer 1994;9:207–15.
5. Mertens F, Rydholm A, Brosjö O, Willén H, Mitelman F, Mandahl N. Hibernomas are characterized by rearrangements of chromosome bands 11q13–21. Int J Cancer 1994;58:503–5.
6. Dei Tos AP, Dal Cin P. The role of cytogenetics in the classification of soft tissue tumors. Virchows Arch 1997;431:83–94.
7. Mandahl N, Mertens F, Willén H, Rydholm A, Brosjö O, Mitelman F. A new cytogenetic subgroup in lipomas: loss of chromosome 16 material in spindle cell and pleomorphic lipomas. J Cancer Res Clin Oncol 1994;120:707–11.
8. Pedeutour F, Forus A, Coindre J-M, Berner J-M, Nicolo G, Michiels J-F, Terrier P, Ranchere-Vince D, Collin F, Myklebost O, Turc-Carel C. Structure of the supernumerary ring and giant rod chromosomes in adipose tissue tumors. Genes Chromosomes Cancer 1999;24:30–41.
9. Antonescu CR, Elahi A, Humphrey M, Lui MY, Healey JH, Brennan MF, Woodruff JM, Jhanwar SC, Ladanyi M. Specificity of *TLS-CHOP* rearrangement for classic myxoid/round cell liposarcoma: absence in predominantly myxoid well-differentiated liposarcomas. J Mol Diagn 2000;2:132–8.
10. Mandahl N. Methods in solid tumor cytogenetics. In: Rooney, DE. Human cytogenetics: malignancy and acquired abnormalities, 3rd ed. New York: Oxford University Press, 2001. 165–203.
11. Mitelman F, ed. ISCN an International System for Human Cytogenetic Nomenclature. Basel: S Karger, 1995.
12. Mandahl N, Heim S, Arheden K, Rydholm A, Willén H, Mitelman F. Three major cytogenetic subgroups can be identified among chromosomally abnormal solitary lipomas. Hum Genet 1988;79:203–8.
13. Mandahl N, Åkerman M, Åman P, Dal Cin P, De Wever I, Fletcher CDM, Mertens F, Mitelman F, Rosai J, Rydholm A, Sciot R, Tallini G, et al. Duplication of chromosome segment 12q15–24 is associated with atypical lipomatous tumors: a report of the CHAMP collaborative study group. Int J Cancer 1996;67:632–5.
14. Mandahl N, Mertens F, Willén H, Rydholm A, Kreicbergs A, Mitelman F. Nonrandom pattern of telomeric associations in atypical lipomatous tumors with ring and giant marker chromosomes. Cancer Genet Cytogenet 1998;103:25–34.
15. Gisselsson D, Höglund M, Mertens F, Johansson B, Dal Cin P, Van den Berghe H, Earnshaw WC, Mitelman F, Mandahl N. The structure and dynamics of ring chromosomes in human neoplastic and non-neoplastic cells. Hum Genet 1999;104:315–25.
16. Dumollard J-M, Ranchére-Vince D, Burel F, Coindre J-M, Tallini G, Ligon AH, Mayaud R, Turc-Carel C, Martin C, Mosnier J-F, Pedeutour F. Lipomes à cellules fusiformes et délétion 13q: apport de la cytogénétique dans l′aide au diagnostic. Ann Pathol 2001;21:303–10.
17. Sciot R, Debiec-Rychter M, De Wever I, Hagemeijer A. Angiomyxolipoma shares cytogenetic changes with lipoma, spindle cell/pleomorphic lipoma and myxoma. Virchows Arch 2001;438:66–9.
18. Höglund M, Sidén T, Åman P, Mandahl N, Mitelman F. Isolation and characterization of radiation hybrids for human chromosome 12. Cytogenet Cell Genet 1995;69:240–5.
19. Jones PA, Laird PW. Cancer epigenetics comes of age. Nat Genet 1999;21:163–7.
20. Kerangueven F, Allione F, Noguchi T, Adélaïde J, Sobol H, Jacquemier J, Birnbaum D. Patterns of loss of heterozygosity at loci from chromosome arm 13q suggest a possible involvement of *BRCA2* in sporadic breast tumors. Genes Chromosomes Cancer 1995;13:291–4.
21. Maestro R, Piccinin S, Doglioni C, Gasparotto D, Vukosavljevic T, Sulfaro S, Barzan L, Boiocchi M. Chromosome 13q deletion mapping in head and neck squamous cell carcinomas: identification of two distinct regions of preferential loss. Cancer Res 1996;56:1146–50.
22. Lin Y-W, Sheu J-C, Liu L-Y, Chen C-H, Lee H-S, Huang G-T, Wang J-T, Lee P-H, Lu F-J. Loss of heterozygosity at chromosome 13q in hepatocellular carcinoma: identification of three independent regions. Eur J Cancer 1999;35:1730–4.
23. Chen C, Frierson Jr HF, Haggerty PF, Theodorescu D, Gregory CW, Dong J-T. An 800-kb region of deletion at 13q14 in human prostate and other carcinomas. Genomics 2001;77:135–44.
24. Wolf S, Mertens D, Schaffner C, Korz C, Döhner H, Stilgenbauer S, Lichter P. B-cell neoplasia associated gene with multiple splicing (*BCMS*): the candidate B-CLL gene on 13q14 comprises more than 560 kb covering all critical regions. Hum Mol Genet 2001;10:1275–85.
25. Shaughnessy Jr J, Tian E, Sawyer J, Bumm K, Landes R, Badros A, Morris C, Tricot G, Epstein J, Barlogie B. High incidence of chromosome 13 deletion in multiple myeloma detected by multiprobe interphase FISH. Blood 2000;96:1505–1511.
26. Borg Å, Zhang Q-X, Alm P, Olsson H, Sellberg G. The retinoblastoma gene in breast cancer: allele loss is not correlated with loss of gene protein expression. Cancer Res 1992;52:2991–4.
27. Cooney KA, Wetzel JC, Merajver SD, Macoska JA, Singleton TP, Wojno KJ. Distinct regions of allelic loss on 13q in prostate cancer. Cancer Res 1996;56:1142–1145.
28. Bièche I, Lidereau R. Loss of heterozygosity at 13q14 correlates with *RB1* gene underexpression in human breast cancer. Mol Carcinogenesis 2000;29:151–8.
29. Zhang X, Xu H-J, Murakami Y, Sachse R, Yashima K, Hirohashi S, Hu S-X, Benedict WF, Sekiya T. Deletions of chromosome 13q, mutations in *Retinoblastoma 1*, and retinoblastoma protein state in human hepatocellular carcinoma. Cancer Res 1994;54:4177–82.
30. Li FP, Abramson DH, Tarone RE, Kleinerman RA, Fraumeni JF, Boice Jr JD. Hereditary retinoblastoma, lipoma and second primary cancers. J Natl Cancer Inst 1997;89:83–4.
31. Mertens D, Wolf S, Schroeter P, Schaffner C, Döhner H, Stilgenbauer S, Lichter P. Down-regulation of candidate tumor suppressor genes within chromosome band 13q14.3 is independent of the DNA methylation pattern in B-cell chronic lymphocytic leukemia. Blood 2002; 99:4116–21.
32. Rowntree C, Duke V, Panayiotidis P, Kotsi P, Palmisano GL, Hoffbrand AV, Foroni L. Deletion analysis of chromosome 13q14.3 and characterisation of an alternative splice form of *LEU1* in B cell chronic lymphocytic leukemia. Leukemia 2002;16:1267–75.
33. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, Fields P, Marshall G, et al. Localization of a breast cancer susceptibility gene, *BRCA2*, to chromosome 13q12–13. Science 1994;265:2088–90.
34. Petit MMR, Schoenmakers EFPM, Huysmans C, Geurts JMW, Mandahl N, Van de Ven WJM. *LHFP*, a novel translocation partner gene of *HMGIC* in a lipoma, is a member of a new family of *LHFP*-like genes. Genomics 1999;57:438–41.