

## CYTOGENETIC STUDY OF BENIGN MESENCHYMAL NEOPLASIAS

C. Casartelli<sup>1</sup>, M.A.M. Ruzzene<sup>1</sup>, S.R. Rogatto<sup>2</sup>, J. Barbieri Neto<sup>3</sup> and  
P.M.P. Philbert<sup>4</sup>

### ABSTRACT

Three human uterine leiomyomas were cultured and analyzed cytogenetically. Although the modal chromosome number was in the diploid range, all the neoplasias had hyperdiploidy. One of the cases had 27% of the cells in the hypertriploid-hypertetraploid range.

The most frequent numerical alterations were monosomies involving chromosomes 20 (in all three of the cases) and 2, 17, 18 (2 cases each), and one case had polysomies of all chromosomes (ranging from trisomy to pentasomy). One case had a large ring chromosome similar to chromosome 1, and a marker with the rearrangement: t(2;12) (2qter → 2q13::12q14-15 → 12pter).

The significance of cytogenetic alterations in benign tumors has yet to be determined.

### INTRODUCTION

Leiomyomas are the most frequent benign uterine neoplasias. Their importance is due to their frequency, clinical manifestations, unfavourable influence on reproduction and the eventual possibility of sarcomatous transformation (Baruffi, 1985).

Because benign tumors generally have no mitotic figures or atypical nuclei, they were considered for many years to be cytogenetically normal. Before the introduction of banding techniques, only sporadic chromosomal studies of benign

---

<sup>1</sup> Departamento de Genética, Faculdade de Medicina, USP, 14049 Ribeirão Preto, SP, Brasil. Send correspondence to C.C.

<sup>2</sup> Departamento de Biologia Geral, Universidade Estadual de Londrina, 86051 Londrina, PR, Brasil.

<sup>3</sup> Departamento de Patologia, Faculdade de Medicina, USP, 14049 Ribeirão Preto, SP, Brasil.

<sup>4</sup> Departamento de Ginecologia, Faculdade de Medicina, USP, 14049 Ribeirão Preto, SP, Brasil.

neoplasias were published (Sandberg, 1980). More recent investigations have demonstrated that karyotypic alterations may characterize neoplastic cells in general (Heim and Mitelman, 1987). Studies on chromosome changes and their associated clinical and histological consequences in both benign and malignant tumors probably could lead to a better understanding of the role of such alterations and tell us much about the frontiers that separate benign lesions from their borderline and malignant counterparts.

We report herein a chromosomal study of three uterine leiomyomas.

### MATERIAL AND METHODS

Leiomyomas from three patients submitted to hysterectomy were sectioned and fragments of the specimens were received under sterile conditions. The material was treated with 0.8% collagenase (IV, Sigma) and placed in culture flasks containing Ham F-10 medium (DIFCO) supplemented with 10% fetal calf serum and antibiotics. For cytogenetic analysis, cells in the exponential growth phase were treated with 0.0016% colchicine for approximately 6 hours, submitted to the action of 0.075 M KCl for 20 minutes and fixed in methanol-acetic acid (3:1). Slides were prepared for standard chromosome analysis and the GTG banding technique was used (Scheres, 1972).

### RESULTS

CASE 1 - Chromosome counts were performed on 46 cells and the modal chromosome number was 46.

Chromosome number	Number of cells
37	01
40	01
42	01
43	01
44	02
45	06
46	30
48	01
50	01
52	01
88	01
Total	<hr/> 46

The most frequent numerical alterations were monosomies of chromosomes 9 and 16 (Figure 1). Less frequent were the monosomies of chromosomes 17, 18, 19, 20, 22 and trisomy of chromosome 14. An unidentified sub-metacentric marker chromosome was present in 17% of the cells.

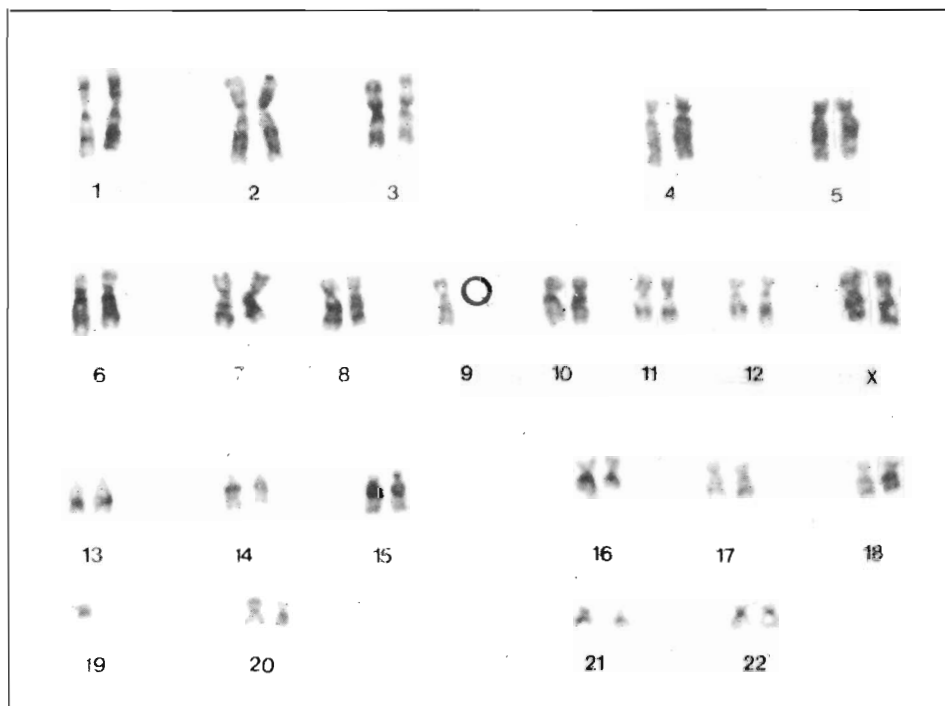


Figure 1 - Karyotype of a cell with 45 chromosomes from case 1. There is a monosomy of chromosome 9.

CASE 2 - Chromosome counts were carried out on 100 cells and the modal number was 46.

Chromosome number	Number of cells
41	03
42	01
43	03
44	02
45	11
46	23

Continued

Continued

Chromosome number	Number of cells
47	13
48	08
49	02
50	03
51	01
52	02
65	01
80	01
81	01
82	01
83	01
84	01
85	01
88	01
89	05
90	03
91	01
92	03
93	03
94	03
95	01
167	01
Total	<hr/> 100

Although the modal chromosome number was 45, 40% of the cells were hyperdiploid, 27% of these being in the hypertriploid-hypertetraploid range. Polysomies involved all chromosomes (Figure 2). Monosomy of 2, 3, 5, 7, 14, 15, 17, 18, 20 was infrequent and there was nullisomy of chromosomes 11, 13, 19, 21 and X.

There was a metacentric marker of unidentified origin in 27% of the cells. Sporadic deletions: 1p-q-, 3p- and 11q- were also found.

CASE 3 - Chromosome counts were performed on 72 cells and the modal number was 45.

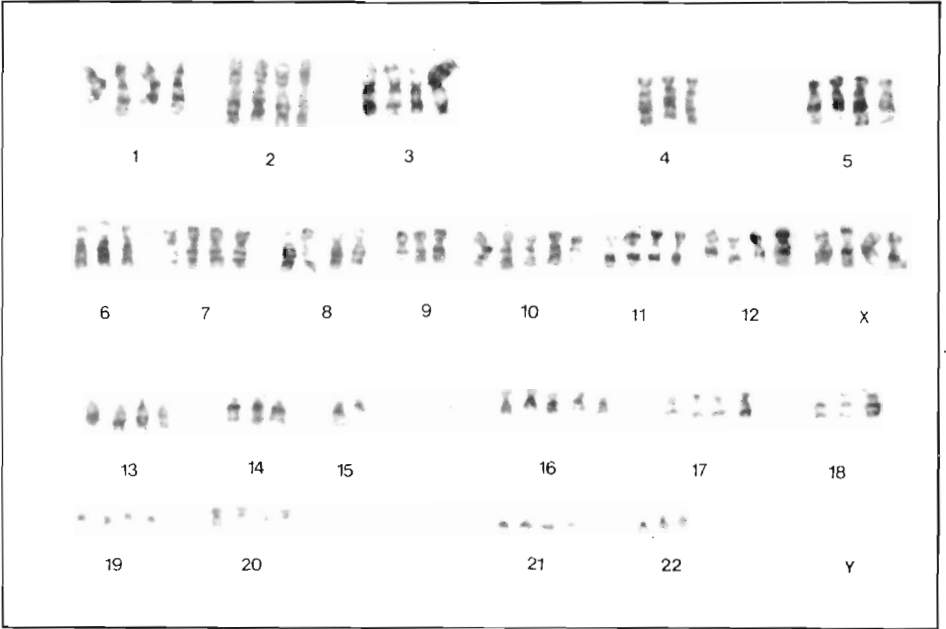


Figure 2 - Karyotype of a cell with 87 chromosomes from case 2. The numerical alterations range from trisomies to pentasomies.

Chromosome number	Number of cells
36	02
37	01
38	02
39	04
40	06
42	04
43	04
44	13
45	16
46	10
47	05
48	02
60	01
>60	02
<b>Total</b>	<b>72</b>

Monosomies of chromosomes 1, 2 and 20 were the most frequent numerical alterations. Sporadic monosomies, trisomies, triradials and fragments were also found.

GTG banding showed a large ring chromosome, resembling chromosome 1, occurring in 88% of the cells (Figure 3); in 65% of the cells analyzed, the following rearrangement was found:  $t(2;12)$  ( $2qter \rightarrow 2q13::12q14-15 \rightarrow 12pter$ ) (Figure 4). An unidentified acrocentric chromosome, similar in size to a D group chromosome, but with a different banding pattern, occurred in 22% of the cells.

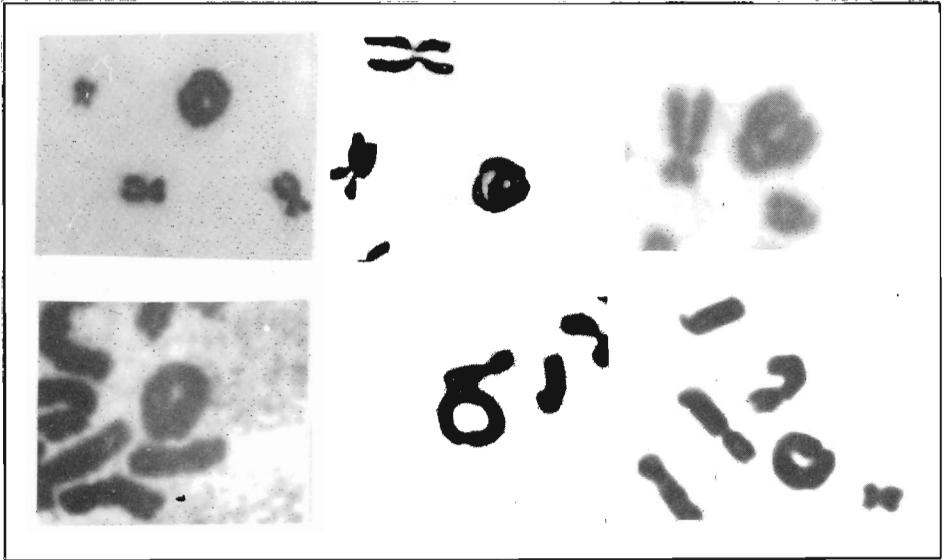


Figure 3 - Some of the ring chromosomes from case 3.

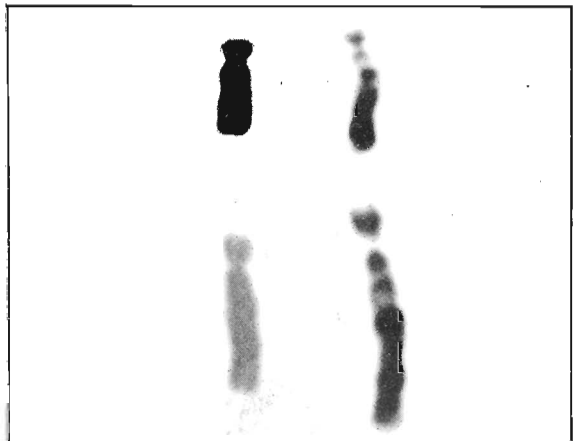


Figure 4 - Marker chromosome  $t(2;12)$  ( $2qter \rightarrow 2q13::12q14-15 \rightarrow 12pter$ ) from case 3.

## DISCUSSION

The presence of clonal karyotypic changes has been demonstrated in benign neoplasias. Meningiomas (Zang, 1982), adipose tumors (Turc-Carel *et al.*, 1986a; Mandahl *et al.*, 1987) and adenomas of the salivary glands (Mark and Dahlenfors, 1986; Bullerdiek *et al.*, 1987) have been well documented.

Reports of chromosome analysis in uterine leiomyomas began to appear in 1988 (Boghosian *et al.*, 1988; Gibas *et al.*, 1988; Heim *et al.*, 1988; Mugneret *et al.*, 1988; Nilbert *et al.*, 1988a,b,c; Turc-Carel *et al.*, 1988; Vanni and Lecca, 1988; Sait *et al.*, 1989; Vanni *et al.*, 1989). The most consistent cytogenetic alteration found in these neoplasias was: t(12;14) (q14-15; q23-24). Other translocations involving this same region of chromosome 12 were found with different partners: chromosomes 22 (Gibas *et al.*, 1988) and X (Vanni and Lecca, 1988). The translocation reported here for case 3 has the same breakpoint on chromosome 12 and the partner is chromosome 2.

The involvement of the 12q13-15 region in structural chromosome alterations in other types of benign tumors including lipomas (Heim *et al.*, 1986; Turc-Carel *et al.*, 1986a; Mandahl *et al.*, 1987) and pleomorphic adenomas (Mark and Dahlenfors, 1986; Bullerdiek *et al.*, 1987) as well as malignant liposarcomas (Turc-Carel *et al.*, 1986b) suggests that a gene or genes related to the neoproliferative cell process may be present near or at this region. It may be of relevance that these breakpoints are near a rare fragile site (12q13.1) (Yunis, 1986) and near the oncogenes INT1 (12q12-13) (Turc-Carel *et al.*, 1987; Arheden *et al.*, 1988) and GLI1 (12q13-q14.3) (Kinzler *et al.*, 1987), although we do not know if the 12q breakpoints are identical in these tumors. This alteration of chromosome 12 was proposed to be associated with a subclass of uterine leiomyomas or perhaps with a subgroup of benign neoplasias. The short arm of chromosome 12 was also reported to be involved in chromosome alterations in leiomyomas (12p11 and 12p13) (Turc-Carel *et al.*, 1988; Vanni *et al.*, 1989). The 12p12 and 12p13 breakpoints were also described in leiomyosarcomas (Nilbert *et al.*, 1988c).

Both chromosomes 12 and 14 have been proposed to be specific for leiomyomas. Among these tumors, there are some with both chromosomes involved in the same rearrangement (Heim *et al.*, 1988; Turc-Carel *et al.*, 1988; Nilbert *et al.*, 1988a; Sait *et al.*, 1989), some with both breakpoints but involved in different alterations (Gibas *et al.*, 1988), some with rearrangements in the 14q22-24 region only (Mugneret *et al.*, 1988), some only with rearrangements in the 12q14-15 region (Gibas *et al.*, 1988; Vanni and Lecca, 1988; Vanni *et al.*, 1989; the present paper) and others with no involvement of these chromosomes in alterations. The oncogene FOS has been localized within the region 14q22-24 (Barker *et al.*, 1984).

In the tumors reported here, chromosome 14 was only involved in numerical alterations: in one case there was trisomy (case 1) and in another monosomy (case 2).

In the present study, there was a high frequency of a ring similar to chromosome 1 in case 3. Nilbert *et al.* (1988b), observed a ring of chromosome 1 in one leiomyoma: r(1) (p34q32). They considered this alteration, as well as t(1;1) (p31q32) which they found in another leiomyoma, to be secondary events in neoplastic cells containing t(12;14). In case 3 reported here, although the possible primary rearrangement was t(2;12), the ring was also present, reinforcing this hypothesis.

Chromosome 7 was in monosomy in our case 2. In leiomyomas, deletions have been found in this chromosome affecting the breakpoints 7q11, 7q21-22 and 7q31 (Boghossian *et al.*, 1988; Turc-Carel *et al.*, 1988; Nilbert *et al.*, personal communication). The breakpoint 7q11 was also involved in a rearrangement in leiomyosarcomas (Nilbert *et al.*, 1988c).

In two of our cases (1 and 2) there was monosomy of chromosome 17. Nilbert *et al.* (1988b) also found a monosomy of this chromosome (although nonclonal). Structural alterations of chromosome 17 were found in leiomyomas, with a breakpoint 17p11 (Gibas *et al.*, 1988) and an iso 17q (Nilbert *et al.*, 1988a). Involvement of 17p13 was found in a leiomyosarcoma (Dal Cin *et al.*, 1988a).

Case one had monosomy of chromosome 22. Other leiomyomas with the same alteration have been reported (Gibas *et al.*, 1988; Turc-Carel *et al.*, 1988) as well as leiomyosarcomas (Dal Cin, 1988b; Nilbert *et al.*, 1988c; Sait *et al.*, 1988). Structural rearrangements of this chromosome involving 22q11-q13 regions were also described in leiomyomas (Gibas *et al.*, 1988; Turc-Carel *et al.*, 1988).

Case 2 presented a large number of numerical alterations, with 27% of the cells in the hypertriploid - hypertetraploid range. Few structural changes were seen in this case, most of them were nonclonal. Nilbert *et al.* (1988b) describe a leiomyoma with two main clones: one of them a near-diploid and the other a hypotetraploid. This tumor had t(12;14) as well as t(1;1).

Different types of chromosome abnormalities in uterine leiomyomas might be related to different histological and/or clinical subgroups. Only by increasing the number of cytogenetic studies and correlating them with the clinical and histopathological picture, could we obtain a better understanding of the meaning of such alterations and determine if any of them might be related to malignant transformation.

Case 3 reported here, with consistent structural rearrangements, had increased cellularity, with 4 mitoses per 10 HPF, when compared to cases 1 and 2 (1 and 0 mitoses per 10 HPF, respectively).

Perhaps cases like this may represent tumors with a very low grade of malignancy, cured by the hysterectomy.

Only in cases of incomplete removal of the tumor without hysterectomy could we be able to correlate the cytogenetic picture with recurrence and/or transformation.

## ACKNOWLEDGMENTS

This work was supported by CNPq, FINEP, FAPESP and CAPES. The authors are grateful to Ms. Vanderci Massaro and Mr. Márcio Rogério Penha for technical help, to Ms. Maria Helena Mamede da Costa for the photography work, to Ms. Susie Adriana Ribeiro Penha Nalon for typing the manuscript and to Dr. Margarida M.F.S. Moraes for making her records available to us.

Publication supported by FAPESP.

## RESUMO

Três leiomiomas uterinos humanos foram cultivados e analisados citogeneticamente. Embora o número modal estivesse na região diplóide, todas as neoplasias apresentaram hiperdiploidia.

Um dos casos apresentou 27% das células na região hipertriplóide-hipertetraplóide.

As alterações numéricas mais frequentes foram monossomias envolvendo os cromossomos 20 (3 casos) e 2, 7, 18 (2 casos cada) e um caso apresentou polissomias de todos os cromossomos (variando de trissomia a pentassomia). Um caso apresentou um grande anel cromossômico semelhante ao cromossomo 1 e um marcador com o rearranjo:  $t(2;12)(2qter \rightarrow 2q13::12q14-15 \rightarrow 12pter)$ .

O significado das alterações citogenéticas em tumores benignos ainda está por ser determinada.

## REFERENCES

- Arheden, K., Mandahl, N., Strömbeck, B., Isaksson, M. and Mitelman, F. (1988). Chromosomal localization of the human oncogene INT1 to 12q13 by *in situ* hybridization. *Cytogenet. Cell Genet.* 47: 86-87.
- Barker, P.E., Rabin, M., Watson, M., Breg, W.R., Ruddle, F.H. and Verma, I.M. (1984). Human c-fos oncogene mapped within chromosomal region 14q21-q31. *Proc. Natl. Acad. Sci. USA* 8: 5826-5830.
- Baruffi, I. (1985). *Tratado de Oncologia togoginecológica e Mamária*. Livraria Roca Ltda., São Paulo.
- Boghosian, L., Dal Cin, P. and Sandberg, A.A. (1988). An interstitial deletion of chromosome 7 may characterize a subgroup of uterine leiomyoma. *Cancer Genet. Cytogenet.* 34: 207-208.
- Bullerdiek, J., Bartnitze, S., Weimberg, M., Chilla, R., Haubrich, J. and Schloot, W. (1987). Rearrangements of chromosome region 12q13-15 in pleomorphic adenomas of the human salivary gland (PSA). *Cytogenet. Cell Genet.* 45: 187-190.
- Dal Cin, P., Boghosian, L., Crickard, K. and Sandberg, A.A. (1988a).  $t(10;17)$  as the sole chromosome change in a uterine leiomyosarcoma. *Cancer Genet. Cytogenet.* 32: 263-266.
- Dal Cin, P., Boghosian, L. and Sandberg, A.A. (1988b). Cytogenetic findings in leiomyosarcoma of the small bowel. *Cancer Genet. Cytogenet.* 30: 285-288.
- Gibas, Z., Griffin, C.A. and Emanuel, B.S. (1988). Clonal chromosome rearrangements in a uterine myoma. *Cancer Genet. Cytogenet.* 32: 19-24.
- Heim, S., Mandahl, N., Kristofferson, U., Mitelman, F., Rööser, B., Rydholm, A. and Willén, H. (1986). Reciprocal translocation  $t(3;12)(q27;q13)$  in lipoma. *Cancer Genet. Cytogenet.* 23: 301-304.
- Heim, S. and Mitelman, F. (1987). *Cancer cytogenetics*. Alan R. Liss, New York.

- Heim, S., Nilbert, M., Vanni, R., Floderus, U.-M., Mandahl, N., Liedgren, S., Lecca, U. and Mitelman, F. (1988). A specific translocation, t(12;14) (q14-15; q23-24), characterizes a subgroup of uterine leiomyomas. *Cancer Genet. Cytogenet.* 32: 13-17.
- Kinzler, K.W., Bigner, S.H., Bigner, D.D., Trent, J.M., Law, M.L., O'Brien, S.J., Wong, A.J. and Volgestein, B. (1987). Identification of an amplified highly expressed gene in a human glioma. *Science* 236: 70-73.
- Mandahl, N., Heim, S., Johansson, B., Bennett, K., Mertens, F., Olsson, G., Rööser, B., Rydholm, A., Willén, H. and Mitelman, F. (1987). Lipomas have characteristic structural chromosomal rearrangements of 12q13-q14. *Int. J. Cancer* 39: 685-688.
- Mark, J. and Dahlenfors, R. (1986). Cytogenetic observations in 100 human benign pleomorphic adenomas. Specificity of the chromosomal aberrations and their relationship to sites of localized oncogenes. *Anticancer Res.* 6: 299-308.
- Mugneret, F., Lizard-Nacol, S., Volk, C., Cuisenier, J., Collin, F. and Turc-Carel, C. (1988). Association of breakpoint 14q23 with uterine leiomyoma. *Cancer Genet. Cytogenet.* 34: 201-206.
- Nilbert, M., Heim, S., Mandahl, N., Floderus, U.-M., Willén, H. and Mitelman, F. (1988a). Karyotypic rearrangements in 20 uterine leiomyomas. *Cytogenet. Cell Genet.* 49: 300-304.
- Nilbert, M., Heim, S., Mandahl, M., Floderus, U.-M., Willén, H., Akerman, M. and Mitelman, F. (1988b). Ring formation and structural rearrangements of chromosome 1 as secondary changes in uterine leiomyomas with t(12;14) (q14-15; q23-24). *Cancer Genet. Cytogenet.* 36: 183-190.
- Nilbert, M., Mandahl, N., Heim, S., Rydholm, A., Willén, H., O'Kerman, M. and Mitelman, F. (1988c). Chromosome abnormalities in leiomyosarcomas. *Cancer Genet. Cytogenet.* 34: 209-218.
- Sait, S.N.J., Dal Cin, P. and Sandberg, A.A. (1988). Consistent chromosome changes in leiomyosarcoma. *Cancer Genet. Cytogenet.* 37: 47-50.
- Sait, S.N.J., Dal Cin, P., Ovanessoff, S. and Sandberg, A.A. (1989). A uterine leiomyoma showing both t(12;14) and del (7) abnormalities. *Cancer Genet. Cytogenet.* 37: 157-161.
- Sandberg, A.A. (1980). *The chromosomes in human cancer and leukaemia*. Elsevier, New York.
- Scheres, Vac M.J.C. (1972). Identification of two Robertsonian translocations with a Giemsa banding technique. *Hum. Genet.* 15: 253-256.
- Turc-Carel, C., Limon, J., Dal Cin, P., Rao, V., Karakousis, C. and Sandberg, A.A. (1986a). Cytogenetic studies of adipose tumors: I. A benign lipoma with reciprocal translocation t(3;12) (q28;q14). *Cancer Genet. Cytogenet.* 23: 283-290.
- Turc-Carel, C., Limon, J., Dal Cin, P., Rao, V., Karakousis, C. and Sandberg, A.A. (1986b). Cytogenetic studies of adipose tissue tumors: II. Recurrent reciprocal translocation t(12;16) (q13;p11) in myxoid liposarcomas. *Cancer Genet. Cytogenet.* 23: 291-299.
- Turc-Carel, C., Pietrzak, E., Kakati, S., Kinniburgh, a.J. and Sandberg, A.A. (1987). The human int-1 gene is located at chromosome region 12q12-12q13 and is not rearranged in myxoid liposarcoma with t(12;16) (q13;p11). *Oncogene Res. I:* 397-405.
- Turc-Carel, C., Dal Cin, P., Boghosian, L., Terk-Zakarian, J. and Sandberg, A.A. (1988). Consistent breakpoints in region 14q22-24 in uterine leiomyoma. *Cancer Genet. Cytogenet.* 32: 25-31.
- Vanni, R. and Lecca, U. (1988). Involvement of the long arm of chromosome 12 in chromosome rearrangements of uterine leiomyoma. *Cancer Genet. Cytogenet.* 32: 33-34.

- Vanni, R., Nieddu, M., Paoli, R. and Lecca, U. (1989). Uterine leiomyoma cytogenetics. I. Rearrangements of chromosome 12. *Cancer Genet. Cytogenet.* 37: 49-54.
- Yunis, J.J. (1986). Chromosomal rearrangements, genes and fragile sites in cancer: clinical and biologic implications. In: *Important Advances in Oncology* (De Vita, V.T. Jr., Helman, S. and Rosenberg, S.A., eds.). J.B. Lippincott, Philadelphia.
- Zang, D.K. (1982). Cytological and cytogenetical studies on human meningioma. *Cancer Genet. Cytogenet.* 6: 249-274.

(Received November 29, 1989)