Vol. 207, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 6, 1995 Pages 363-368

RFLP ANALYSIS OF HUMAN CHROMOSOME 11 REGION q13 IN MULTIPLE SYMMETRIC LIPOMATOSIS AND MULTIPLE ENDOCRINE NEOPLASIA TYPE 1-ASSOCIATED LIPOMAS

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Received December 16, 1994

Six lipomas from patients affected by Multiple Symmetric Lipomatosis (MSL) and by Multiple Endocrine Neoplasia Type 1 (MEN 1) were analyzed for loss of heterozygosity on chromosome 11 region q12-13 using four RFLPs. Allelic loss for the D11S146 locus was found only in one visceral MEN 1-associated lipoma. Lipomas that exhibited a lack of allelic lesions were analyzed for an eventual abnormal amount or a defective function of the Gs protein by studying the Gsα subunit gene, codons 201 and 207, by PCR and TGGE techniques. All the samples were negative for activating mutations.

MSL syndrome is a rare inherited disease clinically characterized by a massive development of large symmetric unencapsulated lipomas on the subcutaneous tissue mainly localized in the neck and in the upper trunk. Pathologic fat accumulation is due to an increase in cell number rather than cell size by a zonal differentiation of adipoblasts into mature adipocytes [1-4]. The typical metabolic abnormalities in this syndrome are an elevated lipoprotein lipase activity in the adipose tissue and the hyperalfalipoproteinemia [5].

MEN 1 syndrome is an autosomal dominantly inherited disorder involving hyperplastic/neoplastic lesions of parathyroid glands, anterior pituitary, and gastro-enteric endocrine cells. Besides these "typical" endocrine localizations, adrenal cortical hyperplasia and adenomas [6], thyroid lesions [6], carcinoid tumors [7], and lipomas [6] have been frequently described in MEN 1 patients. Cutaneous and visceral lipomas constitute the only non-endocrine tumors associated with 10% of patients affected by MEN 1 syndrome [6].

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0006-291X/95 $5.00

These tumors are frequently multicentric [8]. We had the opportunity to investigate for allelic loss on chromosome 11q12-13, where the *men 1* locus has been mapped [9], in six lipoma tissues, four cutaneous from patients with MSL and two, one cutaneous and one visceral (retropharyngeal) associated with familial MEN 1 syndrome. We also performed PCR amplification of Gsα gene, codons 201 and 207, followed by TGGE from five lipomas, one from a MEN 1 patient (the one with allelic loss) and four from MSL patients.

MATERIALS AND METHODS

Patients

Diagnosis of familial MLS and MEN 1 syndromes was performed using already published criteria [10-11]. The lipomas from four MLS patients were obtained from the Geriatric Unit, Internal Medicine Institute, University of Padua. The MEN 1-associated lipomas were from patients followed at the Endocrine Unit, Department of Physiopathology, University of Florence and Gastroenterology and Medical Clinical Institute, University of Bologna.

Chromosome 11q RFLPs and PCR Analysis

All the tissues and EDTA blood samples were frozen at surgery and kept at -80° C. DNA extraction from tissues and peripheral blood was performed as already described [12-13]. Five micrograms of DNA were digested to completion according the manufacturer's instructions and size fractioned by electrophoresis on 0.8 to 1.4% agarose gel. Transfer to Nytran filters (Gene Screen plus, Du Pont, Boston, MA) was performed according to the manufacturer's instructions. Samples were analyzed with four RFLPs that map close the *men 1* gene [9], PYGM (probe pMCMP1, Msp I; Taq I), D11S97 (pMS51, Taq I), D11S146 (pHBI59, Taq I), and INT2 (pSS6, Taq I). The PYGM locus shows recombination 0 with the *men 1* locus [14]. Probes were labelled with $dCTP^{32}$ to a specific activity of approximately 10^9 cpm/μg, with the random priming method. The conditions of prehybridization, hybridization and washing were performed using the manufacturer's specifications (Du Pont, Boston, MA). Filters were autoradiographed at -70° C for 24 to 72 hours. Allelic loss was scored when one expected band in the tumor was completely missing or markedly reduced in intensity compared to the similar band in constitutional DNA. We also performed PCR analysis of the DNA from tissues and matched peripheral blood using oligonucleotide primers, JHE 222 and JHE 224, complementary to a unique sequence flanking a repetitive element, D11S533, telomeric on chromosome 11q [15]. The experimental conditions were carried out according to the original report [15]. We defined the heterozygous state by running a 2% agarose gel and ethidium bromide staining.

PCR Amplification of Genomic DNA and TGGE Techniques

Exons 8 (containing Arg 201) and 9 (containing Gln 227) of the Gsα gene were amplified by PCR using 1 μg of genomic DNA as previously described [16]. Samples were analyzed on 6% acrylamide gels in TBE (Novex) to confirm amplification and then amplified by TGGE (temperature gradient gel electrophoresis) as previously described [17]. TGGE was performed using a commercial apparatus (Diagen, Düsseldorf, Germany). Samples were placed in a sample buffer (final concentration: 20mM MOPS-NaOH, pH 8.0, 5 mM EDTA, 0.1% bromophenol blue, 2% glycerol). 4 μl of each PCR product was analyzed in 5.5% acrylamide (70:1 acrylamide:bisacrylamide) gels containing 20 mM MOPS-NaOH, pH 8.0, 1mM EDTA, 2% glycerol with a running buffer of 20 mM MOPS-NaOH, pH 8.0, 1mM EDTA. Samples were run in a gradient of 30 to 65° C for a total of 70 minutes. Positive controls (provided by J. Lyons) were pituitary tumors containing Arg 201 to Cys (8M1), Arg 201 to His (8M2), Gln 227 to Arg (9M1) and Gln 227 to Leu (9M2). Gels were silver stained.

RESULTS

All six patients were heterozygous for at least one chromosome 11q polymorphism. Only one visceral MEN 1-associated lipoma showed loss of heterozygosity for the D11S146 locus (Fig. 1). A detailed deletion map of the whole lipomas is shown in Table 1. None of the lipomas analyzed by PCR and TGGE for activating mutations in the Gsα gene, codons 201 and 207, resulted to be positive (Fig. 2). We had not enough DNA to perform TGGE analysis of the MEN 1-associated cutaneous lipoma (the one without any appreciable loss of heterozygosity).

DISCUSSION

Recent ultrastructural studies have not shown any differences between lipomatous and normal adipose tissue [3]. It has been hypothesized that lipomatous masses result from trygliceride accumulation following an intrinsic defect in adrenergic stimulated lipolysis [1-4]. This altered response could be due to an abnormal amount or a defective function of Gs-protein, or alternatively, to a defect that could be in the catalytic unit of adenylate cyclase [3-4]. .We now exclude activating mutations in the Gsα gene are involved in the pathogenesis of lipomatous tissue growth. Allelic losses in the *men 1* region was exhibited not only in both MEN 1 and sporadic parathyroid tumors [18-19], but also in uremic hyperparathyroidism [20], and pancreatic tumors [21], while pituitary tumors associated with MEN 1 only rarely show allelic loss at 11q13 chromosome [22-23]. Non MEN 1-associated tumors can also exhibit loss of the heterozygous state for DNA markers in chromosome 11q13, (i.e. human thyroid [24], uterine tumors [25] and, the above mentioned

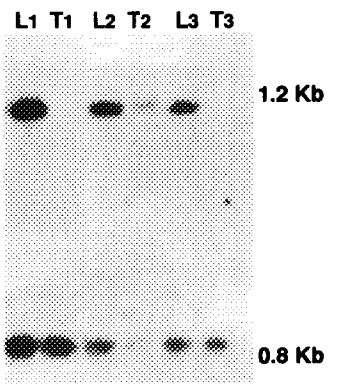

FIGURE 1. RFLP analysis of DNA from MEN 1-associated visceral lipoma (T1), MSL-associated lipoma (T2) and uremic parthyroid gland (T3) (positive control [18]). The filter was hybridized to probe pHBI59 (TaqI), locus D11S146. L1, L2 and L3 represent, respectively, the constitutive leukocite DNA for each tissue. 1.2 and 0.8 Kb indicate the molecular weight of the detected alleles. The allelic loss is scored as a missing band of 1.2 Kb in T1 and the positive control T3. Two flanking sequences, D11S97 (pMS51, TaqI) on centromeric side and INT2 (pSS6, TaqI) on the telomeric side, were uninformative, so we couldn't have a better definition of the allelic loss extension.

TABLE I

Schematic representation of RFLPs analysis of lipomas DNA and their relative matched peripheral blood DNA

DNA	CHROMOSOME 11q LOCI				
	PYGM	D11S97	D11S146	INT2	D11S533
I^	A1-A1	1-1	1-2	1-2	1-2
P. B.	A1-A1	1-1	1-2	1-2	1-2
II*	1-2	1-1	1-*	1-1	3-4
P. B.	1-2	1-1	1-2	1-1	3-4
III	A2-A3	1-1	2-2	1-2	4-4
P. B.	A2-A3	1-1	2-2	1-2	4-4
IV	A1-A3	1-2	2-2	1-2	2-3
P. B.	A1-A3	1-2	2-2	1-2	2-3
V	A2-A3	1-2	2-2	2-2	2-4
P. B.	A2-A3	1-2	2-2	2-2	2-4
VI	A1-A2	1-2	1-1	2-2	2-4
P. B.	A1-A2	1-2	1-1	2-2	2-4

I^: Cutaneous MEN 1-associated lipoma.
II*:Visceral MEN 1-associated lipoma.
III-VI:MSL-associated lipoma.
P.B.: Matched peripheral blood.

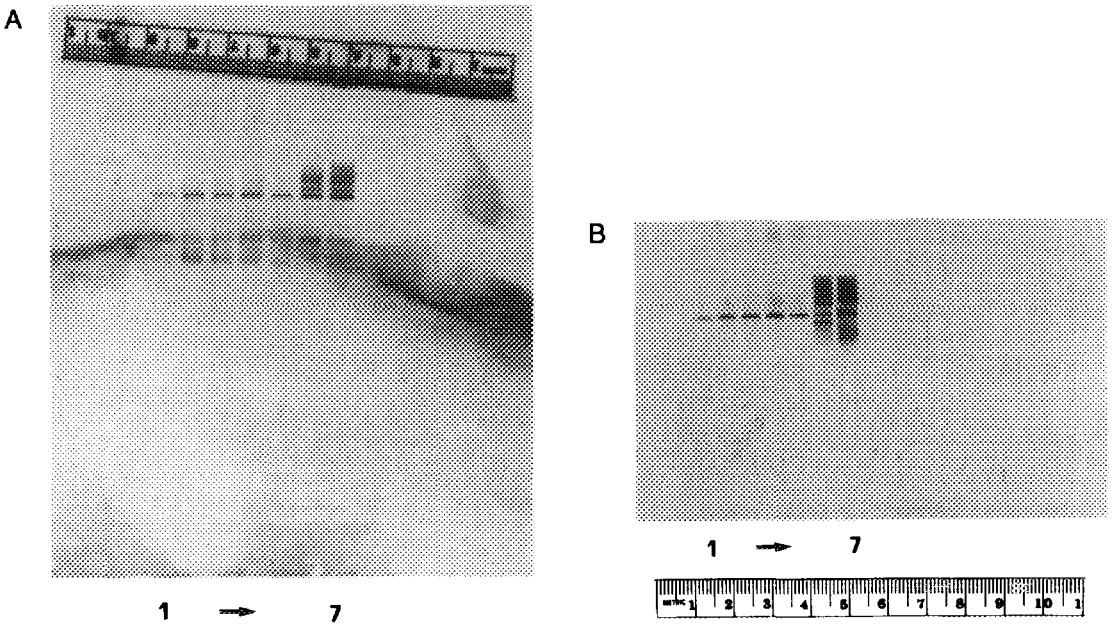

FIGURE 2. Analysis of genomic DNA from five lipomas (see results) for mutations of Gs α codons Arg^{201} and Gln^{227}. Genomic fragments encompassing Gsα exon 8 containing codon Arg^{201} and Gsα exon 9 containing codon Gln^{227} were amplified by PCR and analyzed by TGGE as described in Methods. Gels were silver-stained. The TGGE gel analyzing the exon 8 fragments is shown on figure **2A** and the gel analyzing the exon 9 fragments on figure **2B**. For both gels lanes 1-5 are amplified from genomic DNA derived from lipomas, respectively Lane 1= MEN 1-associated visceral lipoma, Lanes 2-5= MSL-associated lipomas. The positive controls are pituitary tumors known to contain the following mutations: Arg^{201} to Cys (A, lane 6); Arg^{201} to His (A, lane 7); Gln^{227} to Arg (B, lane 6); Gln^{227} to Leu (B, lane 7).

tertiary hyperparathyroidism [20]). These data induce to pinpoint chromosome 11q13 as "hot-spot" region for the presence of known putative anti-oncogenes, such as *men 1* and the *He-La* tumor suppressor genes [26]. The allelic loss found into visceral lipoma from one MEN 1 patient provides an indirect evidence of a monoclonal lesion. It is, therefore, possible to propose that the molecular mechanism that underlies this adipocyte clonal outgrowth involves an unmasking of a recessive mutation of a growth suppressor gene at 11q13 region as already described in parathyroid and pancreatic tumors from MEN 1 patients [18-19].

ACKNOWLEDGMENTS

This work was supported by grants from C.N.R. ("Ingegneria Genetica", P.F.99), and from Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.).

REFERENCES

1. Enzi, G., Inelmen, E., Baritussio, A., Dorigo, P., Prosdocimini, M., and Mazzoleni, F. (1977) J. Clin. Invest. 60: 1221-1229.
2. Cinti, S., Enzi, G., Cigolini, M., and Bosello, O. (1983) Ultrastuctural Pathology 5: 145-152.
3. Zancanaro, C. Sbarbati, A., Morroni, M., Carraro, R., Cigolini, M., Enzi, G., and Cinti, S. (1990) Methods Inf. Med. 63: 253-258.
4. Biasi, D., Caramaschi, P., Carletto, A., Baracchino, F., Botto, M., Pacor, M.L., and Bambara, L.M. (1993) Minerva Med. Mar. 84: 135-139.
5. Enzi, G., Favaretto, L., Martini, S., Fellin, R., Baritussio, A., Baggio, G., and Crepaldi, G. (1983) J Lipid. Res. 24: 566-574.
6. Lips, C.J.M., Vasen, H.F.A., and Lamers, C.B.H.W. (1984) CRC Crit. Rev. Oncol. Hematol. 2: 117-120.
7. Duh, Q.Y., Hybarger, C.P., Geist, R., Gamsu, G., Goodman, P.C., Wooding, G.A.W., and Clark, O.H. (1987) Am. J. Surg. 154: 142-147.
8. Croisier, J.C., Azerod, E., and Lubetzki, J. (1971) Sem. Hop. Paris 47: 494-501.
9. Kidd, K.K., Bowcock, A., Schmidte, J., Track, R.K., Ricciuti, F., Hutchings, G., Bale, A., Pearson, P., Willard, H.F., Gelernter, J., Giuffra, L., and Kubzdela, K. (1989) Cytogenet. Cell Genet. 51: 622-947.
10. Enzi, G. (1984) Medicine 63: 56-64.
11. Brandi, M.L. (1991) J. Endocrinol. Invest. 14: 61-72.
12. Gross-Bellard, J., Oudet, P., and Chambon, P. (1973) Eur. J. Biochem. 36: 32-38.
13. Jeanpierre, M. (1987) Nucl. Acids Res. 15: 9615.
14. Nakamura, Y., Larsson, C., Julier, C., Bystrom, C., Skogseid, B., Wells, S., Oberg, K., Carlson, M., Taggart, T., O'Connell, P., Leppert, M., Lalouel, J.M., Nordenskjold, M., and White,R. (1989) Am. J. Hum. Genet. 44: 751-755.
15. Eubanks, J.H., Selleri, L., Hart, R., Rosette, C., and Evans, G.A. (1991) Genomics 11: 720-729.
16. Weinstein, L.S., Shenker, A., Gejman, P.V., Merino, M.J., Friedma, E., and Spiegel, A.M. (1991) N. Engl. J. Med. 325: 1688-1695.
17. Shenker, A., Laue, L., Kosugi, S., Merendino, J.J. Jr, Minegishi, T., and Cutler, G.B. Jr. (1993) Nature 365(6447): 652-654.
18. Friedman, E., Sakaguchi, K., Bale, A.E., Falchetti, A., Streeten, E., Zimering, M., Weinstein, L.S., Mc Bride, W.O., Nakamura, Y., Brandi, M.L., Norton, J.A., and Aurbach, G.D. (1989) N. Engl. J. Med. 321: 213-218.

19. Thakker, R.V., Bouloux, P., Wooding, C., Chotai, K., Broad, P.M., Spurr, N.K., Besser, G.M., and O'Riordan, J.L. (1989) N. Engl. J. Med. 321: 218-224.
20. Falchetti, A., Bale, A.E., Amorosi, A., Bordi, C., Cicchi, P., Bandini, S., Marx, S.J., and Brandi, M.L. (1993) J. Clin. Endocrinol. Metab. 76: 139-144.
21. Larsson, C., Skogseid, B., Oberg, K., Nakamura, Y., and Nordenskjold, M. (1988) Nature 328: 524-526.
22. Bystrom, C., Larsson, C., Blomberg, C., Sandelin, K., Falkmer, U., Skogseid, B., Oberg, K., Verner, S., and Nordenskjold, M. (1990) Proc. Natl. Acad. Sci. USA 87: 1968-1972.
23. Yoshimoto, K., Iwahana, H., Kubo, K., Saito, S., and Itakura, M. (1991) Jpn. J. Cancer Res. Aug. 82: 886-889.
24. Matsuo, K., Tang, S.H., and Fagin, J.A. (1991) Mol. Endocrinol. 5: 1873-1879.
25. Srivatsan, E.S., Misra, B.C., Venugopalan, M., and Wilczynski, S.P. (1991) Am. J. Hum. Genet. 49: 868-877.
26. Shows, T.B., Davis, L.M., Qin, S., and Novak, N.J. (1986) CSH Symp. Quant. Biol. LI: 867-877.