GENES, CHROMOSOMES & CANCER 17:78–87 (1996)

Detection of Chromosomal DNA Gains and Losses in Testicular Germ Cell Tumors by Comparative Genomic Hybridization

W. Michael Korn, Daniel E.M. Olde Weghuis, Ron F. Suijkerbuijk, Ulrich Schmidt, Thomas Otto, Stanislas du Manoir, Ad Geurts van Kessel, Andreas Harstrick, Siegfried Seeber, and Reinhard Becher

The West German Cancer Center, University of Essen, Medical School, Essen, Federal Republic of Germany (W.M.K., U.S., T.O., A.H., S.S., R.B.); Department of Human Genetics, University Hospital Nijmegen, Nijmegen, The Netherlands (D.E.M.O.W., R.F.S., A.G.v.K.); National Institutes of Health, Bethesda, Maryland (S.d.M.)

To extend the results of conventional cytogenetic analysis of testicular germ cell tumors (TGCTs), we applied the new molecular cytogenetic method of comparative genomic hybridization (CGH), which enables the detection of chromosomal imbalances without the need for dividing cells. DNA from 11 TGCTs was studied by CGH. In all tumors examined, gain of 12p, mostly of the whole p arm, could be demonstrated. However, in three tumors, an amplification of 12p material restricted to the chromosomal bands 12p11.2-p12.1 was found. Further fluorescence in situ hybridization (FISH) analysis using a yeast artificial chromosome (YAC) that was previously mapped to that region revealed multiple copies of that chromosomal segment in interphase nuclei of these tumors. This finding is an important clue to the localization of candidate protooncogenes at 12p involved in TGCTs. Gains of small chromosomal regions at 2p, 4q, 6p, and 19p were also detected recurrently. Furthermore, gains of chromosomes 8, 14, 21, and X as well as loss of chromosome 13 were frequent findings. In conclusion, CGH provides new insights into genetic alterations of TGCTs. By using CGH, chromosomal subregions could be identified that may harbor genes involved in the pathogenesis of this malignancy. *Genes Chromosom Cancer 17:78–87 (1996).*

INTRODUCTION

Conventional cytogenetic analysis has detected several nonrandom numerical and structural abnormalities in testicular germ cell tumors (TGCTs). Gain of chromosomal material from the short arm of chromosome 12 has been found to be the most consistent change. It appears as an isochromosome of 12p in more than 80% of the tumors (for reviews, see de Jong et al., 1990; Chaganti et al., 1993), independent of the histologic subtypes of TGCTs (Rodriguez et al., 1992). Also, in i(12p)-negative TGCTs, an overrepresentation of 12p sequences could be demonstrated by fluorescence in situ hybridization (FISH) experiments (Suijkerbuijk et al., 1992, 1993). Thus, it has been assumed that the amplification of 12p material is a crucial step in the pathogenesis of TGCTs (de Jong et al., 1990; Rodriguez et al., 1993). Because in the majority of tumors almost the entire p arm of chromosome 12 was found to be multiplied (Suijkerbuijk et al., 1993), there was no indication of involvement of any subregions of 12p. Very recently, a high amplification of 12p11.2-p12.1-derived sequences in a metastasis of a seminoma was described, and it was speculated that genes, like the gene encoding the parathyroid hormone-related peptide, localized at this particular region may be relevant for tumor progression (Suijkerbuijk et al., 1994).

Apart from 12p amplification, loss of 12q sequences has been discussed to be an important event in the development of TGCTs (Murty et al., 1992; Rodriguez et al., 1992). Moreover, frequent aberrations of chromosomes 1, 5, 6, 7, 9, 11, 12, 16, 17 and 21 could be detected in TGCTs of different histologic varieties (Rodriguez et al., 1992).

Because most of these findings are results of conventional karyotypic analysis, it must be taken into consideration that this method is successful (in terms of detection of abnormal clones) only in about 50% of the cases. In particular, the analysis of seminomas is very limited due to the low growth rate of these tumors in cell culture; abnormal karyotypes could be detected in only 28% of cases (Rodriguez et al., 1992). In general, conventional cytogenetic analysis is limited by the possibility of artifacts caused by selection processes during short-term cell culture. Furthermore, the analysis of only few metaphases may not provide representative information about the real distribution of aberrations

Received January 8, 1996; accepted April 25, 1996.

Address correspondence to Dr. W. Michael Korn, Division of Molecular Cytometry, Department of Laboratory Medicine, Mission Center Building 230, University of California, San Francisco, CA 94143-0808.

in the majority of tumor cells. Moreover, homogeneously stained regions and double-minute chromosomes, suggesting an amplification of unknown genes, are frequent findings in TGCTs (Rodriguez et al., 1992), but their chromosomal origin cannot be assessed by conventional analysis.

To overcome some of the limitations of conventional cytogenetic analysis, we used comparative genomic hybridization (CGH; Kallioniemi et al., 1992; du Manoir et al., 1993), which allows the detection of chromosomal gains and losses in tumor DNA throughout the whole genome without the need for dividing cells. Briefly, identical amounts of differently labeled tumor and normal (control) DNA are hybridized simultaneously to normal metaphase chromosomes. The hybridized DNA fragments are detected by two different fluorochromes. The ratio between the intensities of the fluorescence signals reflects differences in the quantitative representation of chromosomal material in the tumor DNA compared with the normal DNA and is calculated for all chromosomes by using a digital image analysis system.

By using CGH, we were able to identify the subregion of chromosome 12 (12p11.2-12p12.1) that is amplified in primary TGCTs. Interestingly, an amplification at this location has been shown before for a metastasis. Furthermore, previously unknown sites of DNA amplification at chromosome arms 6p and 4q could be mapped.

MATERIALS AND METHODS

Clinical and Histopathological Data

The clinical and histopathological data of 11 primary tumors included in this study are summarized in Table 1. The clinical stage was classified according to the Lugano classification system (Cavalli et al., 1980). Tumor samples were frozen within 1 hour after surgery. Histopathological examination was performed on tumor specimens from the same tumor site according to standard procedures. Tumor areas were localized microscopically in parallel-step sections before DNA extraction from paraffin-embedded, formalin-fixed tissue.

DNA Extraction

High-molecular-weight DNA was extracted from frozen tumor samples by proteinase-K digestion and phenol-chloroform extraction, according to standard protocols (Davis et al., 1986), or by using a commercial DNA purification kit (QIAamp Tissue Kit; Quiagen GmbH, Hilden, Federal Republic of Germany). In one case (HT-15AM), tumor

TABLE 1. Clinical and Histopathological Data

Case	Age (years)	Histology[a]	Clinical stage[b]	Tumor site[c]
HT-15	28	EC	I	Testis
HT-20	34	SE	IIC	Testis
HT-27	15	YS	IIC	Retroperitoneum
HT-30	33	EC	IIB	Testis
HT-32	40	SE	I	Testis
HT-33	36	SE	I	Testis
HT-43	26	SE, EC	IIIA	Testis
HT-44	17	MT	I	Testis
HT-45	17	IT, EC	I	Testis
HT-53	32	SE	I	Testis
LR-T7	35	SE	IIA	Retroperitoneum

[a]EC, embryonal carcinoma; SE, seminoma; YS, yolk sac; MT, mature teratoma; IT, immature teratoma.
[b]Clinical stage according to the Lugano classification system (Cavalli et al., 1980).
[c]Site of tumor sampled for analysis.

areas in 15 μm paraffin sections were localized by using hematoxylin/eosin-stained, parallel-step sections and were scratched off by a scalpel after deparaffinization with xylene and methanol. After incubation with 1 M NaSCN at 37°C overnight and cell lysis with 0.5% Tween 20, proteinase K (Boehringer Mannheim, Federal Republic of Germany) digestion and phenol-chloroform extraction were performed. In this case, also fresh-frozen tumor tissue was analysed (HT-15FT).

In Situ Hybridization

Double-color FISH experiments using a chromosome 12-centromeric alphoid probe, pα12H8, and the 12p-specific yeast artificial chromosome (YAC) 5 were performed with nuclei extracted from paraffin-embedded tumor material, as described previously (Suijkerbuijk et al., 1994). YAC 5 has been mapped between YAC 753f12 and YAC 951h6 (Krauter et al., 1995). More than 100 nuclei have been examined for each case.

CGH

Following the protocol described by du Manoir et al. (1993), 200–400 ng of tumor DNA and normal control DNA were labeled by nick translation with biotin and digoxigenin, respectively. An appropriate concentration of DNAse-I was chosen to achieve a length of DNA fragments of about 500 bp. Identical amounts of labeled tumor DNA and control DNA were mixed together with 50 μg of Cot1-DNA and 50 μg of sonicated salmon testes DNA (Sigma-Aldrich, Deisenhofen, Federal Republic of Germany). In some cases, normal meta-

phase preparations were pretreated with proteinase K (0.2 μg/ml) and were denatured at 70°C in 70% formamide and 2 × SSC (1 × SSC = 0.15 M NaCL and 0.015 M sodium citrate, pH 7.0). After ethanol precipitation, DNA was resuspended in 12 μl of hybridization mixture, yielding a final concentration of 50% deionized formamide, 1 × SSC, and 10% dextran sulphate. DNA was denatured at 75°C for 5 minutes and incubated at 37°C for 20 minutes before it was applied to the metaphase cells. Hybridization was allowed for 4 days. After washing three times for 5 minutes in 50% formamide/2 × SSC at 45°C, washing three times for five minutes in 0.1% SSC at 60°C, and blocking with 2 × SSC/3% bovine serum albumin (Sigma) for 30 minutes at 37°C, immunochemical staining with 5 μg/ml fluorescein isothiocyanate-avidin (FITC; Vector Lab., Burlingame, USA) and with 2 μg/ml antidigoxygenin rhodamine (TRITC; Boehringer Mannheim, FRG) was performed. Chromosomes were counterstained with 0.1 pg/ml 4,6-diamino-2-phenyl-indole (DAPI; Serva, Heidelberg, FRG) and were mounted in antifading buffer.

Digital Image Analysis

Digitized images were acquired by an epifluorescence microscope (Zeiss Axiophot, Jenoi, FRG) equipped with a cooled, charge-coupled device camera (Photometrics, Tucson, AZ; Kodak 1400 chip). Corresponding images were taken for each metaphase and each fluorochrome by using the appropriate fluorescence filter sets. Image processing was carried out by using software that has been described in detail elsewhere (du Manoir et al., 1993, 1995). Briefly, FITC and TRITC fluorescence profiles were calculated along the chromosome. In a second step, ratios of FITC and TRITC profiles were obtained. Chromosomes were identified by using the banding pattern of the DAPI-stained chromosomes. To determine chromosomal gains and losses and to reduce the influence of artifacts, the fluorescence ratio profiles of at least five metaphases were averaged, and average ratio profiles were plotted for each chromosome. In these plots, the central line reflects the most frequent fluorescence ratio observed, whereas the left and right lines reflect a fluorescence ratio (FR) of 0.75 and 1.25, respectively, which are the theoretical values that would be expected in a diploid tumor cell population for monosomy and trisomy of a certain chromosome present in 50% of the cells, respectively. High-level amplifications were arbitrarily defined for an FR exceeding 2.5.

RESULTS

Average ratio profiles from CGH experiments with DNA from 11 tumors were calculated. The profile of tumor HT-45 is shown in Figure 1. Based on the average ratio profiles, the copy number changes detected by CGH analysis are summarized in Figure 2.

All tumors demonstrated amplifications of a part of the total chromosome 12. In five cases, a high-level amplification of the whole short arm of chromosome 12 was detected. Two tumors demonstrated a gain of both arms of the chromosome, but the amplification level of the short arm was significantly higher than that of the long arm. In three tumors, the gain of DNA sequences derived from chromosome 12 was restricted to the chromosomal subregion 12p11.2-p12.1 (Fig. 3). In cases HT-33 and LR-T7, a very high amplification level of this region could be observed. FISH analysis using YAC 5 showed numerous spots in about 20% of interphase nuclei of case HT-33 (Fig. 4), whereas, in case HT-44, four to eight such spots where detected in several of the nuclei.

In ten cases, an overrepresentation of sequences from the X chromosome was detected. Only in one case (HT-44) the short arm of that chromosome was not involved. In the remaining nine cases, there was a consensus region of the amplifications restricted to the band Xp21.

Furthermore, recurrent gains of chromosomal subregions were found (Fig. 5). In two tumors, amplifications of the region 1p33-1pter and 4q12-4q13 were detected. Three tumors showed amplifications of the distal part of 2p. In four tumors, amplifications of parts of chromosome 6 were found. These were localized at 6p11, 6p11-6p22 (high-level amplification), 6p21-6p22, and 6p21-6pter. At 6p11 for cases HT-15 and HT-32 and at 6p21-6p22 for cases HT-32, HT-45, and LR-T7, the amplifications of material from 6p overlapped. Loss of sequences from chromosome 6 was detected in four cases. Although gains were predominantly present at the short arm, the distal part of 6q was involved in all cases with underrepresentations localized at chromosome 6. Five cases showed overrepresentation of the whole chromosome 19, four of them with a marked gain of 19p. Gains of chromosome 20 were found in five cases, and gains of chromosome 21 were found in seven tumors.

DISCUSSION

CGH is a new molecular cytogenetic technique that enables the detection of chromosomal gains

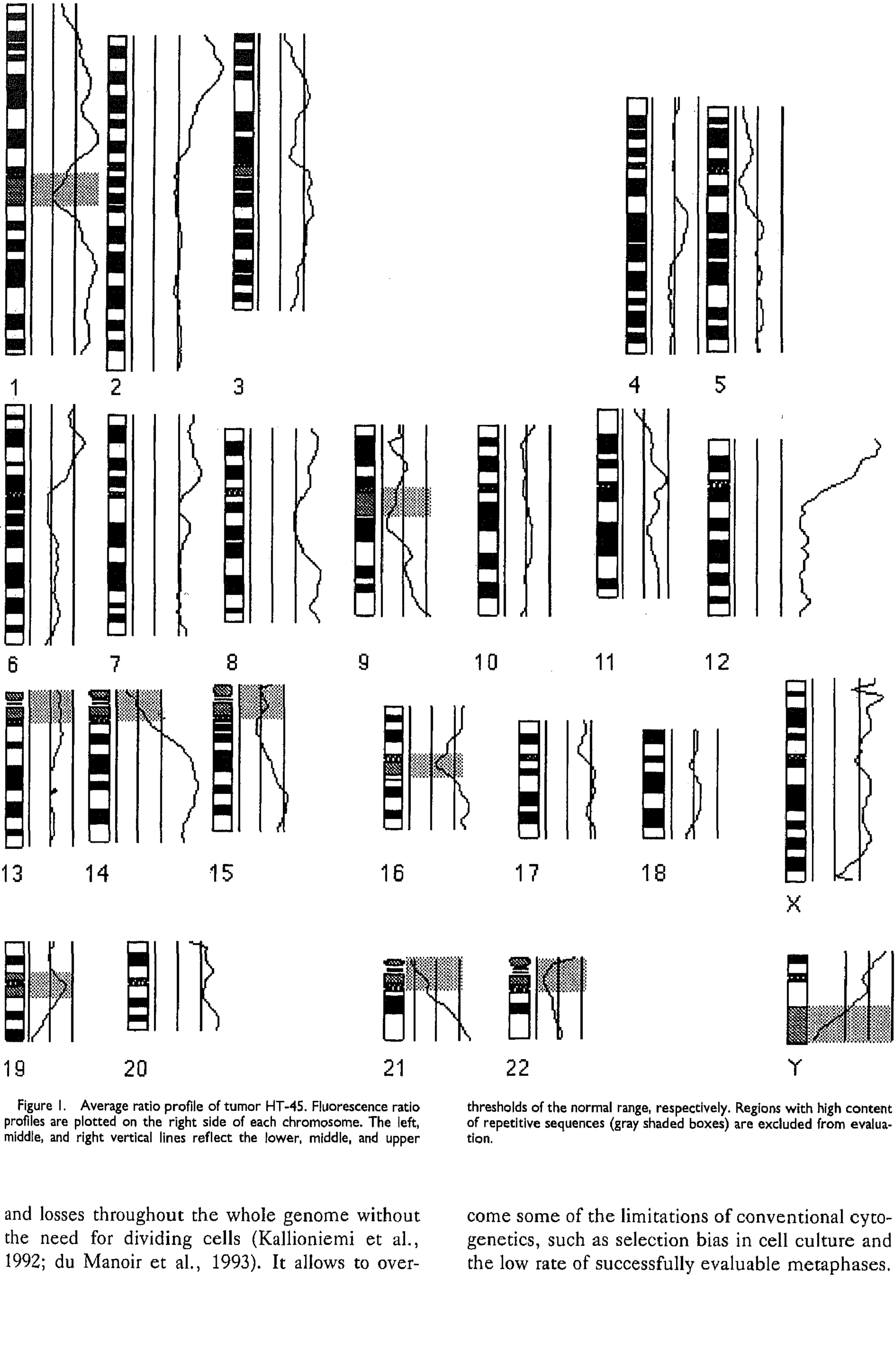

Figure 1. Average ratio profile of tumor HT-45. Fluorescence ratio profiles are plotted on the right side of each chromosome. The left, middle, and right vertical lines reflect the lower, middle, and upper thresholds of the normal range, respectively. Regions with high content of repetitive sequences (gray shaded boxes) are excluded from evaluation.

and losses throughout the whole genome without the need for dividing cells (Kallioniemi et al., 1992; du Manoir et al., 1993). It allows to overcome some of the limitations of conventional cytogenetics, such as selection bias in cell culture and the low rate of successfully evaluable metaphases.

Figure 2. Summary of all gains and losses detected by using comparative genomic hybridization (CGH). The vertical lines on the left side of the chromosome ideograms indicate underrepresentations, and those on the right side indicate overrepresentations of the corresponding chromosomal region. Bold lines reflect high-level amplifications. Case numbers are indicated at the top of the lines.

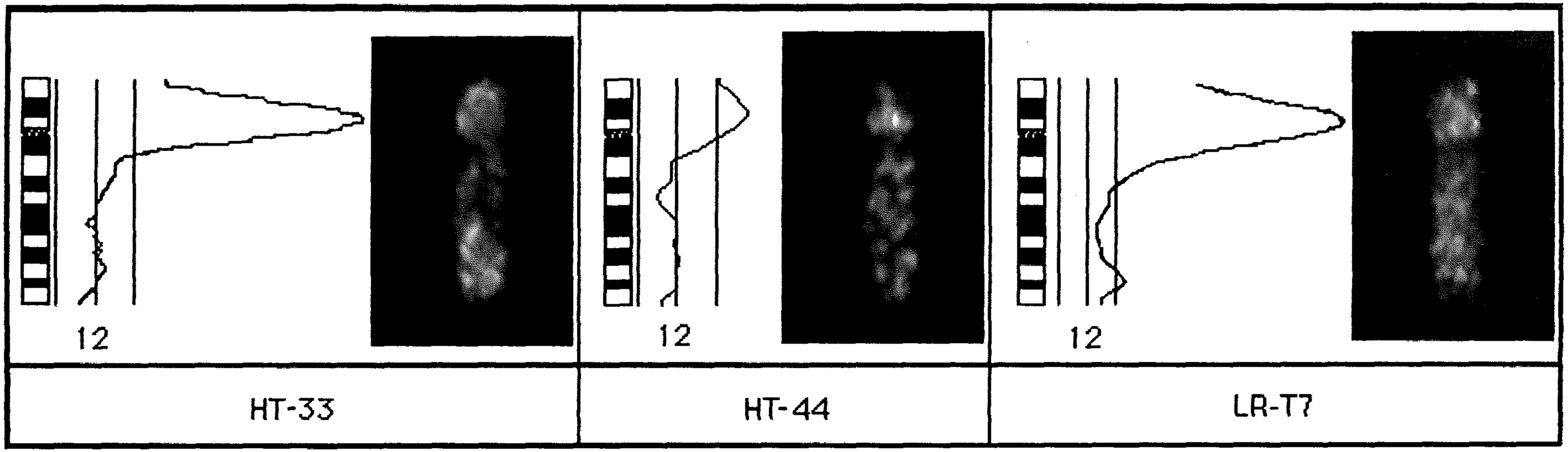

Figure 3. Fluorescence ratio profiles and digital images of normal chromosomes 12 after CGH with tumor DNA from three cases with high-level amplification of the chromosomal region 12p11.2-12p12.1. Tumor DNA was detected by fluorescein isothiocyanate-avidin (FITC; green), and normal control DNA was detected by antidigoxygenin rhodamine (TRITC; red).

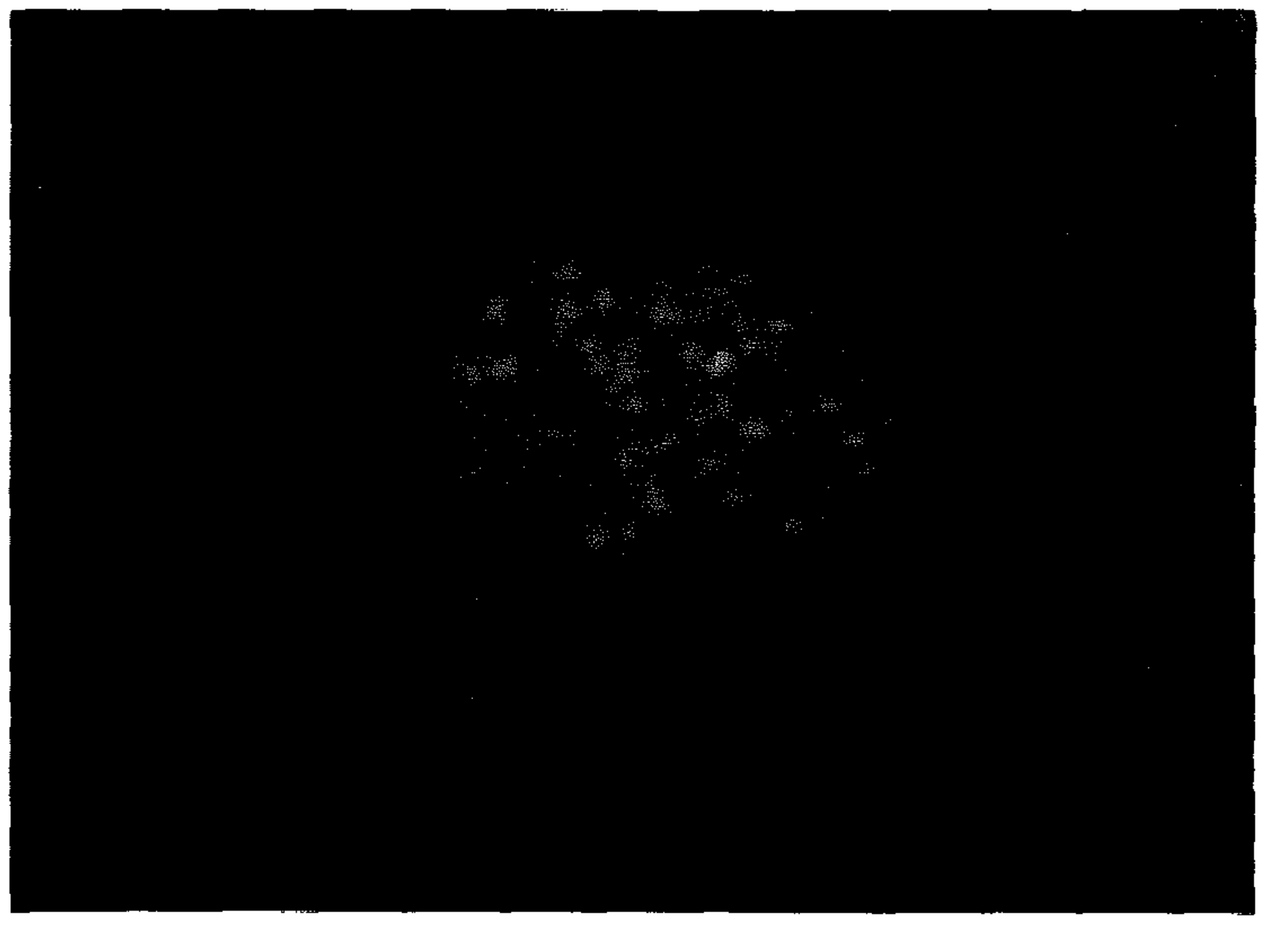

Figure 4. Interphase double-color fluorescence in situ hybridization (FISH) with yeast artificial chromosome (YAC) 5 (green signals) and the chromosome 12-centromeric alphoid probe pα12H8 (red signal) with a nucleus from tumor HT-33.

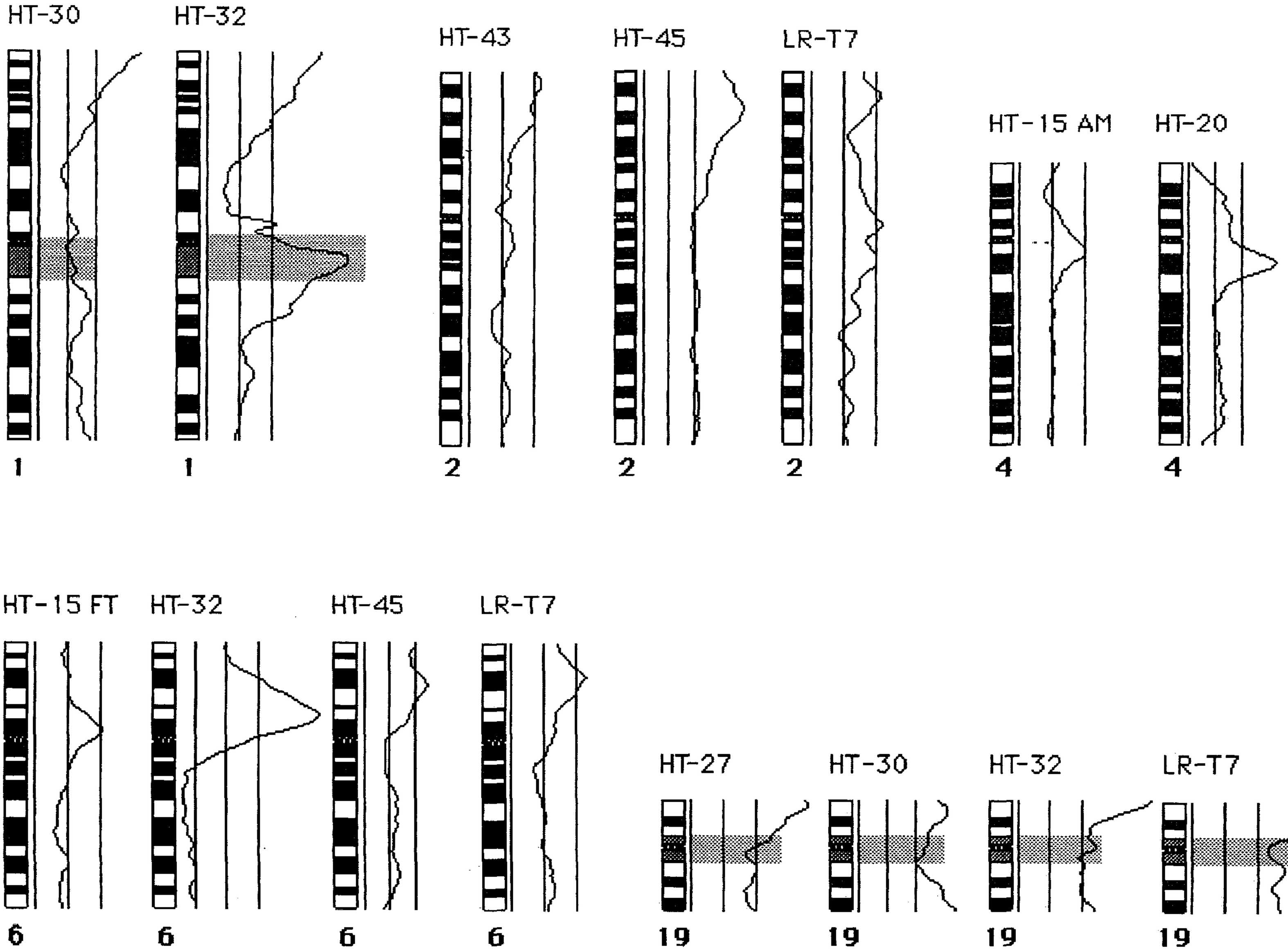

Figure 5. Fluorescence ratio profiles of recurrently detected gains of circumscribed chromosomal subregions. Chromosome numbers are shown at the bottom, and case numbers are indicated at the top of the chromosomes.

Moreover, this method also enables the analysis of archival material (Isola et al., 1994; Speicher et al., 1995).

By using conventional cytogenetic analysis in TGCTs, a variety of recurrent genetic abnormalities have been identified. In most TGCTs, polyploid karyotypes were observed, predominantly in the triploid range, with higher chromosome numbers in seminomas than in nonseminomas (de Jong et al., 1990). Karyotypes and FISH experiments in some of the tumors of this series confirmed the near-triploid status (data not shown). In triploid tumors, it has to be expected that the effect of the loss or gain of one chromosome leads to lower changes of the green-to-red FR than in diploid tumors. Theoretically, the FR in a homogeneous diploid tumor increases from 1.0 to 1.5 in case of a trisomy, whereas the gain of one chromosomal copy results in a triploid tumor in an FR of only 1.33. Because the ploidy status was not determinable for all tumors, the threshold for the detection of gain or loss of one copy was set to 0.75 and 1.25, respectively, following the expected values for diploid tumors. Consequently, a lower sensitivity of the CGH experiments must be considered with a bias towards underestimation of the frequency of low-level copy number changes.

An isochromosome of the short arm of chromosome 12 has been described as a common result in more than 80% of conventional cytogenetic analyses of TGCTs (de Jong et al., 1990; Rodriguez et al., 1992; Chaganti et al., 1993). Also, in i(12p)-negative cases, an amplification of 12p sequences was demonstrated by FISH experiments (Suijkerbuijk et al., 1992, 1993). As in most cases a gain of the whole p arm of that chromosome was found, no hint for the localization of involved genes could be obtained. The present CGH analysis data from 11 TGCTs revealed an overrepresentation of 12p-derived sequences in all tumors analyzed. In the majority of cases, again, the whole p arm of chromosome 12 was found to be amplified, in part at a high level. However, in three cases (HT-33, HT-44, and LR-T7), only the chromosomal subregion 12p11.2-p12.1 was amplified with different levels. In case LR-T7, the amplification of that subregion has been verified by FISH analysis with YAC 5, as described previously (Suijkerbuijk et al., 1994). In the present study, numerous copies of the same chromosomal region were also found in HT-33 by FISH analysis using YAC 5. Because such amplifications detected by CGH have been shown to be a potential correlate of the amplification of protooncogenes (Mohamed et al., 1993), the identification of the 12p-derived amplicon in this tumor may be an important clue to the localization of oncogenes involved in the occurrence of a malignant phenotype in TGCTs. In case LR-T7, because the amplicon was found in DNA from a retroperitoneal lymph node metastasis, an association with tumor progression was assumed (Suijkerbuijk et al., 1994). Histologically, cases HT-33 and HT-44 were characterized as a seminoma and a mature teratoma, respectively, both of which were early-stage primary tumors. Therefore, it is tempting to assume that the amplified genes play an important role in tumor progression independent of the histologic type and clinical stage. It has been suggested that the gene encoding the parathyroid hormone-related polypeptide, which is localized in 12p11.2-p12.1, may be involved in the pathogenesis of TGCTs (Suijkerbuijk et al., 1994). However, our finding of the amplification of this region in a mature teratoma (case HT-44) makes this assumption unlikely, because expression of this gene has been found only in seminomas (Shimogaki et al., 1993).

In 10 of the 11 cases, gain of X-chromosomal material could be detected. With one exception (HT-44), the p arm was involved in all tumors. Xp21 was the only band of the chromosome that was amplified in all of these cases. Recently, a gene from that consensus region was identified that showed high similarity to the *tctex*-1 gene of the mouse t complex (Roux et al., 1994). Because genes localized at that DNA segment play an important role in male germ cell development (Lader et al., 1989), it could be speculated that genes from the corresponding region at the human X chromosome may be involved in the dedifferentiation of germ cells in TGCTs.

Amplifications of parts of chromosomes 1, 2, 4, 6, and 19 were found in at least two cases. The short arm of chromosome 1 is known to be involved frequently in TGCTs. Aberrations with breakpoints at 1p32–36 have been found to be associated with the histology of teratoma (Rodriguez et al., 1992); in the present study, a seminoma and an embryonal carcinoma showed an amplification of this region.

Whereas two tumors demonstrated an overrepresentation of the whole chromosome 2, there was a gain of only the p-terminal part of that chromosome in three other cases. The protooncogene *NMYC* is located at 2p24 and has been found to be highly expressed in seminomas and embryonal carcinomas. However, no DNA amplification has been detected by using Southern blot analysis

(Shuin et al., 1994). Nevertheless, our finding could be the object of further examinations regarding possible amplifications of the *NMYC* gene in these tumors.

In the embryonal carcinoma HT-15 and the seminoma HT-20, amplification of 4q12-q13 was detected. This region is closely localized to the centromere region, which is more difficult to analyze with CGH. This finding could hardly represent an artifact, because the FR profile did not present a variation at the centromere for the other chromosomes in cases HT-15 and HT-20. Also, in a series of uveal melanomas analyzed in the same laboratories, such findings were not observed (Speicher et al., 1994). Interestingly, the gene for the platelet-derived growth factor (PDGF) receptor alpha (*PDGFRA*) has been mapped to 4q11–21 (Hsieh et al., 1991). The PDGF and PDGFRA most likely play important regulatory roles in early embryogenesis (Rappolee et al., 1988; Palmieri et al., 1992). It has been demonstrated recently that, in TGCTs, two novel *PDGRFA* transcripts of 1.5 kilobases and 5.0 kilobases occur, and it was presumed that the smaller one would encode a truncated PDGFA-receptor protein, which might be ligand independent and might have constitutive tyrosine phosphokinase activity (Mosselman et al., 1994, 1996). This putative functional similarity to several protooncogenes might be supported by the high degree of structural homology with the protooncogenes *KIT* and *FMS*. The *KIT* protooncogene has also been mapped to 4cen-q22 (Berdahl et al., 1988) and has been found to be overexpressed in seminomas (Strohmeyer et al., 1991). Further research is necessary to examine whether amplification of the *PDGFRA* gene correlates with the expression of variant *PDGFRA* transcripts and to determine the role of *KIT* in these tumors.

Although aberrations of chromosome arm 6q have been seen nonrandomly in TGCTs (Rodriguez et al., 1992), involvement of the short arm of chromosome 6 has been reported particularly in extragonadal TGCTs (Sinke et al., 1994). In our series, gains or losses of material from that chromosome were detected in 10 of the 11 cases. Interestingly, there was an imbalance of these changes: Whereas DNA gains were found mostly to involve the p arm, loss of DNA was seen particularly at the q arm. Recently, overrepresentation of DNA from 6p was also detected by CGH in osteosarcomas (Forus et al., 1995) and was detected less frequently in breast cancer (Kallioniemi et al., 1994). A candidate protooncogene is *PIM1*, which was mapped to 6p21 (Ziegler et al., 1991). Deletions of the long arm of chromosome 6 were observed recurrently in several solid tumors, particularly in melanoma (Trent et al., 1990), ovarian cancer (Ehlen and Dubeau, 1990), and breast cancer (Devilee et al., 1991). Thus, 6q presumably harbors one or more putative tumor suppressor genes. Based on our findings, it can be speculated that, in TGCTs, overrepresentation of 6p sequences and underrepresentation of DNA from 6q may contribute to tumor development and progression. Although nonrandom aberrations of chromosome 19 have not been observed in TGCTs so far, in three of the cases, high-level amplifications of the short arm of that chromosome were found. However, it has to be considered that the copy number of chromosome 19 sequences tends to be overestimated by CGH.

In accordance with published data from karyotypic analysis (Castedo et al., 1989a,b; De Jong et al., 1990), frequent overrepresentations of chromosomes 7, 8, and 21 were found as well as loss of chromosome 13. This indicates that, in TGCTs, CGH not only allows the detection of new chromosomal copy number changes, but also reflects tumor-typical patterns that are well known from conventional cytogenetics.

In conclusion, by using CGH, we were able to identify a chromosomal subregion of chromosome 12p in primary TGCTs that was found to be highly overrepresented in those tumors and that may harbor genes essential for the pathogenesis of this malignancy. Moreover, several other chromosomal changes were described that should be the basis for further examination, particularly with regard to the role of the *PDGFA* receptor gene at 4q, the putatively involved genes at the proximal part of 6p, and candidate genes at Xp21.

ACKNOWLEDGMENTS

We are indebted to Prof. W. Straube, Essen, and to Drs. F. Dittges and D. Kremer, Dinslaken, for providing the tissue samples. We are grateful to Dr. M.R. Speicher, New Haven, CT, and to Prof. T. Cremer, Munich, for the introduction to CGH and for further advice and support. We thank D. Thyssen and B. Ulrich for their expert technical assistance and Dr. G. Prescher for helpful discussions and critical reading of the paper. Several DNA preparations were kindly carried out by Dr. X.D. Zhang, Peking. This work was supported by Förderverein Essener Tumorklinik e.V., Essen, Federal Republic of Germany.

REFERENCES

Berdahl LD, Murray JC, Besmer P (1988) A *Hind*III RFLP demonstrated for the kit oncogene on chromosome 4. Nucleic Acids Res 16:4740.

Castedo SMMJ, de Jong B, Oosterhuis JW, Seruca R, te Meerman GJ, Dam A, Koops HS (1989a) Cytogenetic analysis of ten human seminomas. Cancer Res 49:439–443.

Castedo SMMJ, de Jong B, Oosterhuis JW, Seruca R, Idenburg VJS, Dam A, te Meerman G, Koops HS, Sleijfer DT (1989b) Chromosomal changes in human primary testicular nonseminomatous germ cell tumors. Cancer Res 49:5696–5701.

Cavalli F, Monfardini S, Pizzocaro G (1980) Report on the international workshop on staging and treatment of testicular cancer. Eur J Cancer 16:1367–1372.

Chaganti RSK, Rodriguez E, Bosl GJ (1993) Cytogenetics of male germ-cell tumors. Urol Clin North Am 20:55–66.

Davis LG, Dibner MD, Battey JF (1986) Basic Methods in Molecular Biology. New York: Elsevier, pp 44–46.

de Jong B, Oosterhuis JW, Castedo SMMJ, Vos A, te Meerman GJ (1990) Pathogenesis of adult testicular germ cell tumors. A cytogenetic model. Cancer Genet Cytogenet 48:143–167.

Devilee P, van Vliet M, van Sloun P, Kuipers-Dijkshoorn N, Hermans J, Pearson PL, Cornelisse CJ (1991) Allelotype of human breast carcinoma: A second major site for loss of heterozygosity is on chromosome 6q. Oncogene 6:1705–1711.

du Manoir S, Speicher MR, Joos S, Schröck E, Popp S, Döhner H, Kovacs G, Robert-Nicoud M, Lichter P, Cremer T (1993) Detection of complete and partial chromosome gains and losses by comparative genomic in situ hybridization. Hum Genet 90:590–610.

du Manoir S, Schröck E, Bentz M, Speicher MR, Joos S, Ried T, Lichter P, Cremer T (1995) Quantitative analysis of comparative genomic hybridization. Cytometry 19:27–41.

Ehlen T, Dubeau L (1990) Loss of heterozygosity on chromosomal segments 3p, 6q and 11p in human ovarian carcinomas. Oncogene 5:219–223.

Forus A, Olde Weghuis D, Smeets D, Fodstad O, Myklebost O, Geurts van Kessel A (1995) Comparative genomic hybridization analysis of human sarcomas: II. Identification of novel amplicons at 6p and 17p in osteosarcomas. Genes Chromosom Cancer 14: 15–21.

Hsieh CL, Navankasattusas S, Escobedo JA, Williams LT, Francke U (1991) Chromosomal localization of the gene for AA-type platelet-derived growth factor receptor (PDGFRA) in humans and mice. Cytogenet Cell Genet 56:160–163.

Isola J, DeVries S, Chu L, Ghazvini S, Waldman F (1994) Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. Am J Pathol 145:1301–1308.

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258: 818–821.

Kallioniemi A, Kallioniemi OP, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Pinkel D, Gray JW, Waldman FM (1994) Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc Natl Acad Sci USA 91: 2156–2160.

Krauter K, Montgomery K, Yoon SJ, LeBlanc-Straceski J, Renault B, Marondel I, Herdman V, Cupelli L, Banks A, Lieman J, Menninger J, Bray-Ward P, Nadkarni P, Weissenbach J, Le Paslier D, Rigault P, Chumakov I, Cohen D, Miller P, Ward D, Kucherlapati R (1995) A second-generation YAC contig map of human chromosome 12. Nature 377:321–333.

Lader E, Hae-Sook H, O'Neill M, Artzt K, Bennett D (1989) *tctex*-1: A candidate gene family for a mouse t complex sterility locus. Cell 58:969–979.

Mohamed AN, Macoska JA, Kallioniemi A, Kallioniemi OP, Waldman F, Ratanatharathorn V, Wolman SR (1993) Extrachromosomal gene amplification in acute myeloid leukemia: Characterization by metaphase analysis, comparative genomic hybridization, and semiquantitative PCR. Genes Chromosom Cancer 8:185–189.

Mosselman S, Claesson-Welsh L, Kamphuis JS, van Zoelen EJJ (1994) Developmentally regulated expression of two novel platelet-derived growth factor α-receptor transcripts in human teratocarcinoma cells. Cancer Res 54:220–225.

Mosselman S, Looijenga LHJ, Gillis AJM, van Rooijen MA, Kraft HJ, van Zoelen EJJ, Oosterhuis JW (1996) Aberrant platelet-derived growth factor α-receptor transcript as a diagnostic marker for early human germ cell tumors of the adult testis. Proc Natl Acad Sci USA 93:2884–2888.

Murty VVVS, Houldsworth J, Baldwin S, Reuter V, Hunziker W, Besmer P, Bosl G, Chaganti RSK (1992) Allelic deletions in the long arm of chromosome 12 identify sites of candidate tumor suppressor genes in male germ cell tumors. Proc Natl Acad Sci USA 89:11006–11010.

Palmieri SL, Payne J, Stiles CD, Biggers JD, Mercola M (1992) Expression of mouse *PDGF-A* and PDGF α-receptor genes during pre- and post-implantation development: Evidence for a developmental shift from an autocrine to a paracrine mode of action. Mech Dev 39:181–191.

Rappolee DA, Brenner CA, Schultz R, Mark D, Werb Z, Ross R, Raines EW, Bowen-Pope DF (1988) Developmental expression of *PDGF*, *TGF*-α, and *TGF*-β genes in preimplantation mouse embryos. Cell 46:155–169.

Rodriguez E, Mathew S, Reuter V, Ilson DH, Bosl GJ, Chaganti RSK (1992) Cytogenetic analysis of 124 prospectively ascertained male germ cell tumors. Cancer Res 52:2285–2291.

Rodriguez E, Houldsworth J, Reuter VE, Meltzer P, Zhang J, Trent JM, Bosl GJ, Chaganti RSK (1993) Molecular cytogenetic analysis of i(12p)-negative human male germ cell tumors. Genes Chromosom Cancer 8:230–216.

Roux AF, Rommens J, McDowell C, Anson-Cartwright L, Bell S, Schappert K, Fishman GA, Musella M (1994) Identification of a gene from Xp21 with similarity to the *tctex*-1 gene of the murine t complex. Hum Mol Genet 3:257–263.

Shimogaki H, Kitazawa S, Maeda S, Kamidono S (1993) Variable expression of hst-1, int-1, and parathyroid hormone-related protein in different histologic types of human testicular germ cell tumors. Cancer J 6:81–86.

Shuin T, Misaki H, Kubota Y, Yao M, Hosaka M (1994) Differential expression of protooncogenes in human germ cell tumors of the testis. Cancer 73:1721–1727.

Sinke RJ, Olde Weghuis D, Suijkerbuijk RF, Tanigami A, Nakamura Y, Larsson C, Weber G, de Jong B, Oosterhuis JW, Molenaar WM, Geurts van Kessel A (1994) Molecular characterization of a recurring complex chromosomal translocation in two human extragonadal germ cell tumors. Cancer Genet Cytogenet 73:11–16.

Speicher MR, Prescher G, du Manoir S, Jauch A, Horsthemke B, Bornfeld N, Becher R, Cremer T (1994) Chromosomal gains and losses in uveal melanomas detected by comparative genomic hybridization. Cancer Res 54:3817–3823.

Speicher MR, Jauch A, Walt H, du Manoir S, Ried T, Jochum W, Sulser T, Cremer T (1995) Correlation of microscopic phenotype with genotype in a formalin-fixed, paraffin-embedded testicular germ cell tumor with universal DNA amplification, comparative genomic hybridization, and interphase cytogenetics. Am J Pathol 146:1332–1340.

Strohmeyer T, Peter S, Hartmann M, Munemitsu S, Ackermann R, Ullrich A, Slamon DJ (1991) Expression of the hst-1 and c-*kit* protooncogenes in human testicular germ cell tumors. Cancer Res 51:1811–1816.

Suijkerbuijk RF, Looijenga L, de Jong B, Oosterhuis JW, Cassiman JJ, Geurts van Kessel A (1992) Verification of isochromosome 12p and identification of other chromosome 12 aberrations in gonadal and extragonadal human germ cell tumors by bicolor double fluorescence in situ hybridization. Cancer Genet Cytogenet 63:8–16.

Suijkerbuijk RF, Sinke RJ, Meloni AM, Parrington JM, van Echten J, de Jong B, Oosterhuis JW, Sandberg AA, Geurts van Kessel A (1993) Overrepresentation of chromosome 12p sequences and karyotypic evolution in i(12p)-negative testicular germ-cell tumors revealed by fluorescence in situ hybridization. Cancer Genet Cytogenet 70:85–93.

Suijkerbuijk RF, Sinke RJ, Olde Weghuis DEM, Roque L, Forus A, Stellink F, Siepman A, van de Kaa C, Soares J, Geurts van Kessel A (1994) Amplification of chromosome subregion 12p11.2-p12.1 in a metastasis of an i(12p)-negative seminoma: Relationship to tumor progression? Cancer Genet Cytogenet 78:145–152.

Trent JM Stanbridge EJ, McBridge HL, Meese EU, Casey G, Araujo DE, Witkowski CM, Nagle RB (1990) Tumorigenicity in human melanoma cell lines controlled by introduction of human chromosome 6. Science 247:568–571.

Ziegler A, Field LL, Skagushi AY (1991) Report of the committee on the genetic constitution of chromosome 6. Cytogenet Cell Genet 58:295–336.