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Comparison of constitutional and tumor-associated 11;22 translocations: Nonidentical breakpoints on chromosomes 11 and 22

(*ETS1*/immunoglobulin light chain/Ewing sarcoma/neuroepithelioma)

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ABSTRACT **Recurring, site-specific chromosomal rearrangements are associated with several human syndromes and malignant disorders. Such nonrandom translocations involving chromosome 22 in band q11 are numerous and found to be associated with a diversity of neoplasms as well as constitutional disorders. Chromosome 11 in bands q23–q24 is similarly involved in several types of tumors as well as in a recurring constitutional reciprocal translocation with chromosome 22. Here we report the use of chromosomal *in situ* hybridization to compare the translocation breakpoints in the cytologically indistinguishable constitutional t(11;22) and the tumor-related t(11;22) associated with Ewing sarcoma and peripheral neuroepithelioma. We have shown that the breakpoints can be distinguished from each other with respect to the locus encoding the constant region of the Ig λ light chain (C_λ) at 22q11 and the *ETS1* locus at 11q23→q24; *ETS1* has been called hu-*ets-1* or human c-*ets-1*. The tumor-associated chromosome 11 breakpoint is also different from those of leukemias with t(9;11) and t(4;11) translocations. Southern-blot analysis showed no rearrangement of *ETS1* in these disorders in the region detected by our probe. *ETS1* has also been mapped more precisely to 11q23.3→q24 by *in situ* hybridization to cells from an individual with an 11q23.3→qter deletion.**

Site-specific chromosome rearrangements are associated with both malignant and nonmalignant human disorders. Recurrent rearrangements involving chromosome 22 have been described both in the constitutional karyotype and as acquired somatic abnormalities in neoplastic diseases (1, 2). The breakpoints of the t(9;22) of chronic myelogenous leukemia, the t(9;22) of acute lymphocytic leukemia, and the t(8;22) of Burkitt lymphoma within 22q11 are cytologically indistinguishable. Chromosomal *in situ* hybridization has been used to map these translocation breakpoints more precisely and has shown that, in fact, the 22q11 breakpoints can be distinguished from each other at a molecular level (3, 4).

Recently, chromosome 22 has been shown to be involved in a reciprocal translocation with chromosome 11, t(11;22)-(q23–q24;q11–q12) in virtually all cases of Ewing sarcoma (ES) and peripheral neuroepithelioma (NE) examined cytogenetically (5–10). Askin tumor, a malignancy of the thoracopulmonary region, also has been found recently to have an apparently identical t(11;22) (10) and is likely a neuroepithelioma of the chest wall (11, 12). Recurrent rearrangements involving chromosome 11 at q23–q24 have also been reported in leukemias, including the t(4;11)(q21;q23) of acute lymphoblastic or undifferentiated leukemia (13) and the t(9;11)(q21;q23) of acute monocytic leukemia (14).

The 11;22 rearrangement of ES, NE, and Askin tumor is cytologically indistinguishable from the recurrent constitutional chromosomal rearrangement that has been described (1), which involves the same chromosomal regions. The constitutional t(11;22)(q23;q11) is a site-specific, reciprocal translocation that has now been described in more than 110 unrelated families (1, 15). Balanced carriers are phenotypically normal and at no apparent increased risk for neoplasia. The translocation is usually detected after the birth of phenotypically abnormal progeny who carry the derivative chromosome 22 as a supernumerary chromosome. This 11;22 translocation is one of the most common reciprocal translocations in the constitutional karyotype of man. While the breakpoints of the rearrangement given by different authors vary between bands q23 and q25 of chromosome 11 and bands q11 and q13 of chromosome 22 (15, 16), they are, in practice, cytologically indistinguishable from each other. Molecular data suggest that the breakpoint is in 22q11 (17); therefore, the complementary breakpoint in chromosome 11 would have to be q23 to give the observed derivative chromosomes.

We have used chromosomal *in situ* hybridization to compare this constitutional 11;22 translocation with the 11;22 translocation associated with ES and NE. Using a portion of the constant (C) region of the immunoglobulin λ light chain gene (C_λ) and the protooncogene *ETS1* (human homolog of the E26 acute avian oncogene *ets-1*, often referred to as hu-*ets-1*) as probes, we have shown that the breakpoints within 22q11 and 11q23 appear to be alike in four constitutional translocation carriers but clearly distinguishable from the cytologically similar breakpoints associated with ES and NE. The *ETS1* locus also has been sublocalized more precisely to the region 11q23.3→q24 by using cells carrying an 11q23.3→qter deletion.

MATERIALS AND METHODS

Cells and Cell Lines. Cells from four unrelated females carrying the balanced t(11;22) translocation were used for *in situ* hybridization studies. Lymphoblastoid cell lines GM6229 and GM6275 were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). A fibroblast cell line, GB, established at The Children's Hospital of Philadelphia provided cells from the third individual, and phytohemagglutinin-stimulated peripheral blood lymphocytes were obtained from a fourth unrelated individual, HD. Phytohemagglutinin-stimulated peripheral blood lymphocytes were obtained from an infant, SH, who had multiple congenital anomalies and a constitutional *de novo* deletion of 11q.

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Abbreviations: ES, Ewing sarcoma; NE, peripheral neuroepithelioma; C, constant; kb, kilobase(s).
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Human tumor cell lines used included NE lines N1000, N1008, N1016 [from National Institutes of Health (M.A.I.)], TC32 (from T. J. Triche, National Institutes of Health), CHP 100 (from The Children's Hospital of Philadelphia), and SKNMC (from J. L. Biedler, Sloan–Kettering Laboratories, New York); and ES lines N1001, N1002 [from National Institutes of Health (M.A.I.)], TC106, TC71, 6674, 5838, A4573 (from T. J. Triche, National Institutes of Health), and ML (from E.C.D. and A.A.G., St. Jude Children's Research Hospital). N1000, N1002, ML, and TC32 were used for *in situ* hybridization studies, and all tumor cell lines except ML were used for DNA studies.

High-resolution chromosomal studies using modified trypsin-Wright staining to produce G-banding (18) were performed on the four constitutional translocation carriers. Karyotypes showed 46,XX,t(11;22)(q23;q11) in each case. The GB line, in addition, had a pericentric inversion of chromosome 9. The karyotype of SH was 46,XX,del(11)-(q23.3→qter) in all cells examined. All tumor lines were verified to possess the t(11;22) translocation in addition to other chromosomal abnormalities as reported (refs. 5 and 10; E.C.D., unpublished data) except N1008, which contains only the derivative chromosome 22 (10).

Probes. pCλ is an 8.0-kilobase (kb) *Eco*RI genomic fragment of the C region of the human immunoglobulin λ light chain in pBR322 (19). It contains the coding regions for $C_{\lambda 2}$ and $C_{\lambda 3}$ and has been shown to map to 22q11 (19, 20). The DNA probe for the human protooncogene *ETS1* is a 5.4-kb *Eco*RI fragment of genomic DNA in plasmid pKH47 (21), which has been shown to map to 11q23→q24, and which represents a portion of the *ETS1* locus.

***In Situ* Hybridization.** ^{3}H-labeling of DNA and *in situ* hybridization were performed by using a protocol modified from several in the literature that has been described in detail (22). Concentrations of probe DNA used were 0.035–0.11 μg/ml. Slides were exposed to Kodak NTB-2 liquid track emulsion for 3–21 days, developed, and stained for G-banding by using a modified Wright Giemsa protocol (23). Seventy-five to 100 metaphase spreads were analyzed from each individual or cell line for each probe. Locations of grains on chromosomes were recorded. Because of the limited technical quality of banding of the preparations from the tumor cells, chromosomes 22, 22q−, 11, and 11q+ were identified individually whenever possible and by group (G and C, respectively) when necessary.

Southern-Blot Analysis. DNA was isolated from tumor-derived cell lines and from normal tissues of the same individual when available by using the procedure of Britten *et al.* (24). Genomic DNA (20 μg) was cleaved with a 5-fold excess of the appropriate restriction enzyme and fractionated by electrophoresis on a 1% agarose gel in Tris acetate buffer (40 mM Tris·HCl, pH 7.5/5 mM sodium acetate/1 mM EDTA) at 25 V overnight. The DNA in the gel was transferred to nitrocellulose filters (Schleicher & Schuell) as described by Southern (25). The *ETS1* probe was labeled with ^{32}P-labeled deoxynucleotides by using a nick-translation kit (Amersham). Hybridization and washing procedures have been described elsewhere (26).

RESULTS

Partial karyotypes showing chromosomes 11 and 22 from two constitutional t(11;22) carriers, one ES cell line, and one NE cell line, are shown in Fig. 1 to illustrate the similarity of the constitutional and tumor-related breakpoints on chromosomes 11 and 22. The terminal deletion in individual SH, del(11)(q23.3→qter) (Fig. 2) is in the same region of 11q as are the constitutional and tumor-related translocations of chromosome 11.

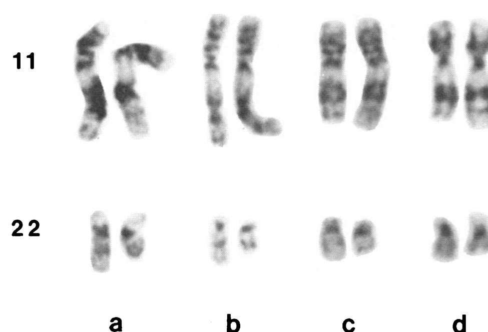

FIG. 1. Partial karyotypes comparing chromosomes 11 and 22 from constitutional t(11;22) carriers GB (*a*) and GM6275 (*b*), NE cell line TC32 (*c*), and ES cell line N1002 (*d*). The derivative chromosome is on the right of each pair of chromosomes. The translocation breakpoints are indistinguishable from each other.

The results of *in situ* hybridization of labeled pCλ DNA to metaphase chromosome preparations from two constitutional t(11;22) carriers are summarized in Table 1. Preliminary results from 26 previously reported metaphases (17) are included in the results for GM6229. In the two constitutional t(11;22) carriers GM6229 and HD, pCλ hybridized to its normal site on chromosome 22 and also to the 11q+ chromosome, with no significant hybridization to the 22q− (derivative chromosome 22). In contrast to the results for the constitutional t(11;22) and similar to previously reported results for the tumor-related rearrangement (17), the pCλ probe hybridized to both the normal and derivative chromosomes 22 in the ES cell line ML, with an additional large percentage of grains on G-group chromosomes, which, due to limited technical quality, could not be identified absolutely in some metaphases. Thus, taken together with previous results, the breakpoint on chromosome 22 is proximal to the C_λ locus in the five constitutional translocation carriers examined and distal to the C_λ locus in the four tumors examined.

In situ hybridization of labeled *ETS1* DNA (Table 2) to metaphase chromosome spreads from the four constitutional t(11;22) carriers showed significant hybridization to the normal site at 11q23→q24 and also to the 22q− chromosome. Thus, *ETS1* translocates to the derivative chromosome 22 in the constitutional t(11;22) translocation. In contrast, there was no significant hybridization to the derivative chromosome 22 (Table 3 and Fig. 3) in either of the two ES cell lines or the two NE cell lines that we examined. Thus, the breakpoint on chromosome 11 is proximal to the *ETS1* locus in the four constitutional translocation carriers examined and distal to *ETS1* in the four tumor-associated 11;22 rearrangements examined.

Ninety-eight metaphases from SH were hybridized with labeled *ETS1* DNA to localize more precisely the *ETS1* locus

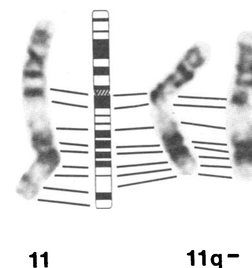

FIG. 2. High-resolution chromosomes 11 from individual SH. A *de novo* constitutional deletion of 11q23.3→qter was present in all cells examined. The normal chromosome 11 is to the left of the ideogram, and two examples of the deleted chromosome 11 are to the right.

Table 1. *In situ* hybridization results with the probe C_λ

t(11;22) cell lines	Metaphases, no.	Total grains analyzed, no.	Grains over chromosomal site,* no.		
			11q+	22	22q−
Constitutional					
HD	100	181	32 (18)	34 (19)	2 (1)
GM6229	75	108	25 (23)	23 (21)	3 (3)
Tumor					
ML	101	199	5 (3)	23 (12)	18 (9)†

*Percentages of total grains are shown in parentheses.
†In addition, 21 grains (11%) were seen on unidentifiable G-group chromosomes.

on distal 11q; 180 chromosomally localized grains were recorded, with 14 grains (7%) on the normal chromosome 11 at 11q23→qter and 3 grains (1.6%) at the terminus of the 11q− chromosome. In two separate experiments in which this *ETS1* probe was hybridized to normal male cells (composite data in Table 2), the mean hybridization to 11q23→qter was 11%, and the range of hybridization, within 2 SEMs, was 6–16%. The hybridization we observed to the 11q− chromosome is outside this range. The limited hybridization of the *ETS1* probe to the deleted chromosome suggests that homologous sequences have been lost because of the deletion. Thus, these results, taken together with the data for the tumor-associated t(11;22), which shows that ETS remains on the involved chromosome 11, suggest that the *ETS1* locus can be sublocalized to 11q23.3→q24.

To determine whether we could identify a rearrangement of *ETS1* in either ES or NE cell lines, we examined DNA isolated from 13 tumor cell lines previously shown to have the t(11;22) translocation. Fig. 4 *Upper* is a representative autoradiograph showing results of a Southern-blot analysis of 6 of these lines. DNA isolated from 3 NE cell lines, normal tissue from the same 3 individuals, and 3 ES cell lines was cleaved with *Apa* I and probed with ^{32}P-labeled *ETS1*. *Apa* I cleaves twice within the region recognized by the probe to produce a 2.3-kb fragment. The 5′ flanking genomic fragment is 9 kb, and the 3′ flanking genomic fragment is 10 kb, as shown in Fig. 4. Since the pattern in the 6 tumor DNAs is identical to that of the 3 DNAs from matched normal tissues, at least 21 kb surrounding the region recognized by this *ETS1* probe are not rearranged by these chromosomal translocations. Other experiments using the enzymes *Eco*RI, *Hin*dIII, and *Bam*HI to digest all of these DNAs also revealed no evidence of rearrangement (data not shown) and extend the region devoid of rearrangement around our *ETS1* probe to 25 kb. However, since the entire *ETS1* locus is probably larger than 25 kb, these experiments do not completely exclude the possibility of a rearrangement within the *ETS1* locus.

DISCUSSION

Chromosome-banding techniques have allowed identification of translocations that occur nonrandomly as acquired abnormalities in an increasing variety of neoplasms. These abnormalities are thought to indicate sites in the human genome where genes important to the development or maintenance of neoplasia are located. The most extensively studied tumor involving an abnormality of chromosome 22 is chronic myelogenous leukemia. The derivative chromosome 22 (the Philadelphia chromosome) is formed by a translocation between chromosomes 9 and 22, t(9;22)(q34;q11) (2), in which the *ABL* (human c-*abl*) oncogene from chromosome 9 is brought adjacent to the breakpoint cluster region on chromosome 22 (27). This results in a chimeric mRNA that produces a fusion protein with acquired protein-tyrosine kinase activity (28–32). The derivative chromosome 22 also occurs in acute lymphocytic leukemia, but the genetic mechanism of *ABL* activation or involvement is less clear (34). Chromosome 22 is also involved in about 10% of the cases of Burkitt lymphoma, with a translocation between chromosomes 8 and 22, t(8;22)(q24;q11) (3, 19). The rearrangement activates the *MYC* (human c-*myc*) oncogene on chromosome 8 as a result of the interruption of the phage λ variable immunoglobulin sequences on chromosome 22 (2, 19). In all three diseases, region 22q11 is involved.

Recurrent site-specific rearrangements of chromosome 22 also occur in the constitutional human karyotype, most frequently in the balanced translocation t(11;22)(q23;q11). While these recurrent rearrangements of chromosome 22, both acquired and constitutional, are all within q11 at the cytologic level, at the molecular level they are due to recombination over sequences perhaps spanning several thousand kilobases. We can postulate that some of these rearrangements are seen recurrently as a result of the λ light chain gene structure, since recognition signals encoded by specific DNA sequences and enzymes to mediate such somatic recombination exist (33), and evidence exists for immunoglobulin gene involvement in the t(8;22) of Burkitt lymphoma. Whether as-yet-unidentified homologous sequences are present on chromosomes 11 and 22 that allow recurrent translocation between them remains to be determined. Comparative molecular mapping of breakpoints within regions that are apparently identical cytologically is necessary to provide a linear order to the breakpoints within the region and to direct the approach to the location of involved DNA sequences.

Translocation breakpoint mapping of the constitutional t(11;22) and the cytogenetically similar translocation of ES, NE, and Askin tumor has allowed us to determine molecular differences between these cytologically indistinguishable translocations. Data has been presented that showed that the breakpoint within 22q11 is proximal to C_λ in three constitutional t(11;22) carriers and distal to C_λ in one ES cell line and two NE cell lines with the t(11;22) translocation (17). We have confirmed and extended these results with one additional constitutional translocation carrier and one additional ES cell line. In a similar fashion, we now can distinguish the breakpoint on chromosome 11 with reference to the *ETS1* gene. We have shown that the breakpoint is proximal to *ETS1*

Table 2. *In situ* hybridization results with probe *ETS1* in constitutional t(11;22)

	Metaphases, no.	Total grains analyzed, no.	Grains over chromosomal site,* no.			
			11q23→qter	11q+	22	22q−
HD	75	182	22 (12)	4 (2)	3 (2)	23 (13)
GB	77	167	13 (8)	3 (2)	3 (2)	18 (11)
GM6229	75	177	14 (8)	5 (3)	5 (3)	21 (12)
GM6275	75	172	11 (6)	3 (2)	3 (2)	27 (16)
Normal male	84	141	16 (11)	—	5 (4)	—

*Percentages of total grains are shown in parentheses.

Table 3. *In situ* hybridization results with probe *ETS1* in tumors with t(11;22)

	Metaphases, no.	Total grains analyzed, no.	Grains over chromosomal site,* no.			
			11q23→qter	11q+	22	22q−
N1002	75	164	12 (7)	14 (9)	5 (3)	0 (0)
ML	80	186	38 (20)	49 (26)	1 (<1)	0 (0)
N1000	75	143	19 (13)	10 (7)	1 (<1)	0 (0)
TC32	107	210	32 (16)	12 (6)	2 (1)	1 (<1)

*Percentages of total grains are shown in parentheses.

in four constitutional t(11;22) carriers and distal to *ETS1* in the four tumors examined (Fig. 5). The *ETS1* locus appears to be located within or distal to 11q23.3 on the basis of our *in situ* hybridization to cells from an individual with a *de novo* deletion in 11q, since we did not find significant hybridization to the 11q− chromosome. It is, however, possible that the *ETS1* gene was interrupted by the translocation and the sequences remaining on the 11q− chromosome are outside the region recognized by our current probe. Unfortunately, neither DNA nor metaphase spreads from this now deceased individual are available to look for rearrangement of the *ETS1* locus.

The cell of origin for ES is unknown; NE is of neuroectodermal origin (11). Recent work by Whang-Peng *et al.* (10) has described a cytologically indistinguishable t(11;22) in 22 cases of ES, 5 cases of NE, and 5 Askin tumors. An additional neuroepithelioma with t(11;22) is described by de Chadarevian *et al.* (6). To date, no site-specific, recurrent chromosomal translocation has yet been described in tumors arising from histologically distinct tissues. Our finding that the breakpoints on chromosomes 11 and 22 for two NE and two ES cell lines are indistinguishable from each other with respect to the location of *ETS1* and C_λ genes lends further support to the similarity of these tumors and credence to the possibility that neuroepithelioma and ES arise from a common precursor cell (10). The chromosome 11 breakpoint of ES and NE cell lines is distal to *ETS1* and, thus, is clearly different from that of two cases of monocytic leukemia with t(9;11)(p22;q23) (14) and of one case of t(4;11) leukemia (13) reported, in which the breakpoints are proximal to *ETS1*.

The role of oncogenes in 11;22 translocations, both constitutional and tumor-related, remains unclear. *SIS* (human c-*sis*), which has been mapped to 22q13 (34) and is therefore far distal to the region of chromosome 22 involved in these translocations, is translocated in ES (35, 36) and NE (37) cell lines, and there is no evidence of activation or rearrangement of *SIS* (35–37). *ETS1* has not been shown to be rearranged in one study of five ES cell lines (21), and we have no evidence of *ETS1* rearrangement in the tumor lines we have studied. In fact, using restriction enzymes which permit us to look both 3′ and 5′ of our genomic probe, a 25-kb area can be excluded from translocation-mediated rearrangement. Further studies of this locus with additional genomic probes as they become available will be required to delineate its role, if any, in the etiology of this group of tumors.

Similarly, a role for *ETS1* in constitutional translocation carriers is unknown and unlikely. In three individuals examined thus far (C.M., M.A.I., M. Budarf, and B.S.E., unpublished data), *ETS1* is not rearranged, suggesting that the translocation breakpoints on chromosome 11 in these cases are outside those sequences detected by our current probe. The translocation of *ETS1* in constitutional t(11;22) carriers provides an example of a situation where translocation of an oncogene does not result in neoplasia, a reminder that

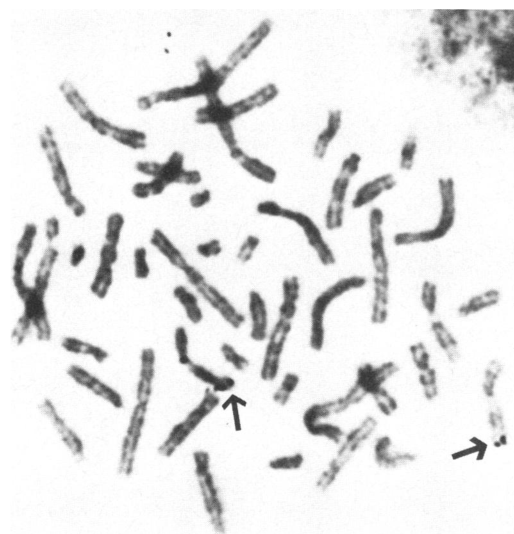

FIG. 3. Representative autoradiograph from *in situ* hybridization with ^{3}H-labeled genomic *ETS1* probe to a metaphase from ES cell line ML. Arrows point to grains on normal and derivative chromosomes 11.

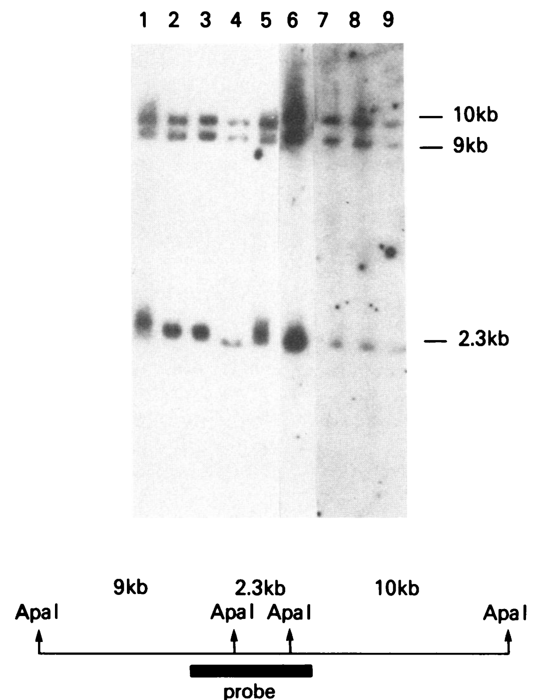

FIG. 4. (*Upper*) Southern blot analysis of normal and tumor DNAs. DNAs from three neuroepithelioma cell lines [N1000 (lane 2), N1016 (lane 4), and N1008 (lane 6)], normal tissue from the same patients, respectively (lanes 1, 3, and 5), and three ES cell lines [TC106 (lane 7), 6647 (lane 8), and TC71 (lane 9)] were cleaved with *Apa* I, electrophoresed, transferred, and hybridized as described. (*Lower*) Map of the region covered by the 5.4-kb genomic *ETS1* probe and the *Apa* I restriction map of the surrounding genomic DNA deduced by hybridization with 3′ and 5′ ends of the probe. All DNA samples showed the germ-line restriction pattern characteristic for this probe.

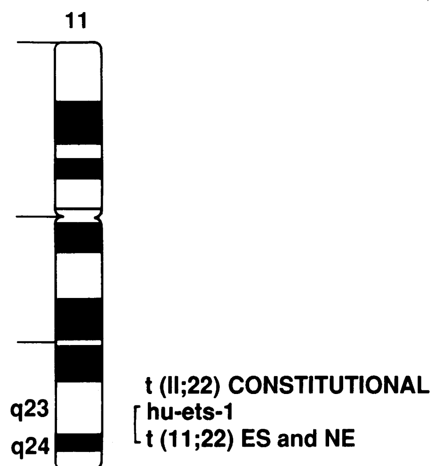

FIG. 5. Diagram of chromosome 11 showing the relative locations of the breakpoints for four constitutional t(11;22) carriers and four tumor-related t(11;22) translocations with respect to the *ETS1* locus (hu-*ets-1*). As indicated, *ETS1* is located either in the distal portion of 11q23 or in the proximal portion of 11q24 and is proximal to the tumor-associated breakpoint.

moving a protooncogene from its normal chromosomal location in the genome is not sufficient in itself to result in neoplastic transformation. Further work will be required to map the breakpoints in constitutional and tumor-related t(11;22) more precisely. *NCAM* (38), *THY1* (38), and *T3D* (39) at 11q23, and *D22S9* (40) at 22q11 are other recently mapped genes that may prove useful as probes for further translocation breakpoint mapping. Ultimately, examination of sequences at or near the breakpoint junctions will be required, both to uncover mechanisms by which these recurrent translocations occur and to define which specific genes, if any, are altered by the translocation.

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