RAPID COMMUNICATION

# Molecular Definition of a Narrow Interval at 7q22.1 Associated With Myelodysplasia

By E.J. Johnson, S.W. Scherer, L. Osborne, L.C. Tsui, D. Oscier, S. Mould, and F.E. Cotter

**Chromosome 7 translocations, deletions, or monosomy are associated with myelodysplasia (MDS) and acute myeloid leukemia both in children and adults. These chromosomal anomalies represent one of the most common cytogenetic abnormalities associated with these diseases and usually herald a poor prognosis. In this study two cosmid DNA probes that mapped to 7q22.1 and were known to be separated by approximately 500 kb were identified to flank the proximal inversion breakpoint in a patient carrying a constitutional inversion (7q22.1-34) associated with MDS. A yeast artificial chromosome (YAC) clone that encompassed the two cosmids was identified and shown to span the breakpoint. Fluorescence in situ hybridization was then used to analyze six additional patients with myelodysplasia and chromosomal rearrangements of the 7q22 region (three patients had translocations and three carried deletions). The breakpoint in one of the patients was found to be contained within the same YAC clone that spanned the inversion breakpoint. Moreover, this same interval was determined to be absent in all three patients with chromosomal deletions. These results suggest that this segment of DNA on chromosome 7q22.1 may contain specific gene(s) that have a significant role in myeloid malignancies.**


MYELODYSPLASIA (MDS) has a bimodal age distribution with peaks in the first and sixth decades. It is characterized by dysplastic and ineffective hematopoiesis and a tendency to progress to acute leukemia. The French-American-British (FAB) classification is used for the adult disease but is not as readily applicable in children because of specific conditions that do not clearly fit the FAB groups. These include juvenile chronic myeloid leukemia (JCML) and infant monosomy 7 syndrome (IMo7), which require certain clinical and cytogenetic features for diagnosis.[1,2] Prognosis may be determined by a number of factors, particularly in childhood MDS, such as platelet count, hemoglobin F (HbF) level, and cytogenetic complexity.

A recent study showed that of 63 cases of childhood MDS in whom karyotype analysis was performed, 55% had clonal abnormalities affecting chromosome 7.[3] Within this group, monosomy 7 seems to be more common than 7q deletions. A review of childhood monosomy 7 has clearly divided the myeloid disorders associated with monosomy 7 into three groups: de novo disorders (MDS, acute myeloid leukemia [AML], monosomy 7 syndrome, and JCML), secondary disorders (therapy-related, occupational exposure and severe aplastic anaemia), and constitutional disorders (Fanconi's anemia, Kostmann's syndrome, Shwachman's syndrome, Neurofibromatosis type 1, Down syndrome, and familial monosomy 7).[4] However, JCML is distinct in that it is very rarely associated with chromosome 7 abnormalities, even at the submicroscopic level.

Loss of heterozygosity of chromosome 7 as determined using polymorphic probes on material from patients with MDS/AML with no detectable cytogenetic abnormality is very uncommon.[5] There is a male predominance in de novo disorders and Shwachman's syndrome but a more equal sex incidence in Kostmann's syndrome and familial monosomy 7.[4] Treatment of these diseases is generally difficult, although chemotherapy can delay the advancement of disease in some instances. However, progression to acute leukemia with monosomy 7 is extremely difficult to treat, with few successes after bone marrow transplantation (BMT).[6]

A tumor-suppressor gene model[7,8] has been proposed, but it is not known if the loss of function of only one allele (haploinsufficiency) is needed to cause disease.[9] Most evidence suggests that chromosome 7 loss is necessary but not sufficient for disease and is a relatively late event in disease progression. This applies to both adult and childhood disease. No candidate gene has yet been identified on chromosome 7. Cytogenetic studies suggest as "critical regions" a commonly involved DNA segment in band 7q22 and less commonly another at 7q32-34.[5,10] Restriction fragment length polymorphism (RFLP) and pulsed-field analysis has been used in an attempt to define deletions in adults with MDS/AML and 7q deletions [termed del(7q)].[11-13] These experimental approaches may be problematic because the chromosomally abnormal cells (which are sometimes present in relatively low numbers) are often mixed in with normal cells. Notwithstanding, the proximal 7q22 breakpoints could be located between the genes for erythropoietin (EPO) and plasminogen activator inhibitor 1 (PLANHI) in four patients in one study[11] and three patients in another[13] (Fig 1). In the latter study, breakpoints for two additional patients were positioned between elastin (ELN) and collagen 1 $\alpha$ 2 (COL1A2)[13] (Fig 1). The critical region of deletion within

*From the LRF Department of Haematology and Oncology, Institute of Child Health, London, UK; the Department of Genetics, The Hospital for Sick Children, Toronto; the Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; and the Department of Haematology, Royal Bournemouth Hospital, UK.*

*Submitted November 2, 1995; accepted February 2, 1996.*

*E.J.J. is supported by an LRF clinical research fellowship, L.-C.T. is supported by Grant No. G012317 from the Canadian Genome Analysis and Technology Program, The Canadian Genetic Disease Network, and the Howard Hughes Medical Institute.*

*Address reprint requests to F.E. Cotter, MD, PhD, LFR Department of Haematology and Oncology, Institute of Child Health, 30 Guilford St, London WC1N 1EH, UK.*

*The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*


*0006-4971/96/8709-0061$3.00/0*

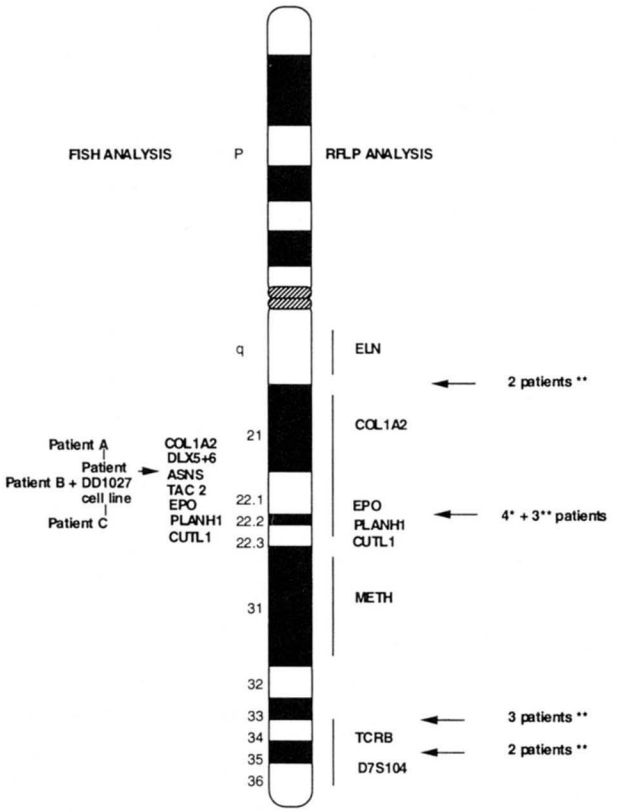



**Fig 1. Chromosome 7 with (left): position of breakpoint in DD1027 cell line and relative positions of breakpoints in translocation patients A, B, and C (Table 2), and (right): positions of breakpoints as reported in RFLP studies by *Kere et al, 1989[11] and by **Lewis et al, 1993.[13]**

7q32-qter occurred proximal to the T-cell receptor $\beta$ gene at 7q35 in two patients and proximal to the anonymous marker D7S104 at 7q36-qter in five patients.[13]

The introduction of fluorescence in situ hybridization (FISH) has allowed for more accurate analyses of rearrangements at the cytogenetic and molecular level.[14] We have applied this approach to try to clarify further the nature of the chromosome changes in MDS and AML. Translocations and inversions affecting the region of interest are less common in MDS but may provide a more accessible means of identifying putative causative genes than monosomy of chromosome 7 and larger deletion samples. FISH with chromosome 7–specific yeast artificial chromosomes (YACs) and cosmids from the q21-22 bands was used to analyze and localize such breakpoint regions.

## MATERIALS AND METHODS

*Cell line and bone marrow (BM) samples.* The Epstein-Barr virus transformed lymphoblastoid cell line DD1027 was obtained from ECACC (Salisbury, UK). Culture was at 37°C and 6% carbon dioxide in RPMI, 10% fetal calf serum, with added glutamine, streptomycin, and tetracycline. A cell concentration of between 0.5 and 2.0 $\times$ $10^6$ was maintained. BM samples were collected in heparin tubes and cultured at 37°C in RPMI 1640 supplemented with 10% 5637 conditioned medium[15] and a concentration of $10^6$ cells/mL.

*Preparation of metaphase spreads.* The unsynchronized BM cultures and DD1027 cell line cultures were arrested at metaphase by the addition of colcemid (0.5 $\mu$g/mL) for 45 minutes and the cells were fixed in 3:1 methanol:glacial acetic acid. Cells were dropped onto glass slides that were then kept at least 24 hours before use. Metaphase chromosomes were banded by standard G-banding with trypsin-Giemsa (GTG)[16] and karyotyped according to the International System for Human Cytogenetic Nomenclature. For FISH, slides were washed in 2 $\times$ standard saline citrate (SSC) for 1 hour, denatured in 70% formamide at 70°C for 2 minutes, then dried in 70%, 85%, and 100% ethanol, at room temperature, 3 minutes in each.

*FISH analysis.* The cosmids and YACs (see Table 1) were grown in appropriate conditions and DNA was prepared using standard procedures and purified using RNase and phenol/chloroform extraction. One microgram of cosmid or YAC DNA per sample was nick-translated with biotin-14-dATP, using the Bionick Labelling System (GIBCO BRL, Paisley, UK). Probe was also prepared by Alu-PCR as described.[17] Both methods of probe preparation gave comparable results. Cot-1 DNA (GIBCO BRL) was added to bind repetitive sequences. A digoxigenin-labeled $\alpha$ satellite chromosome 7 centromere probe (Oncor) and in three cases a biotin-labeled $\alpha$ satellite chromosome 2 probe (Oncor, Gaithersburg, MD) (patients A, B, and C) were added before the labeled cosmids were hybridized to chromosome DNA overnight at 37°C in a humid chamber. Posthybridization washing was performed in 50% formamide, 2 $\times$ SSC, $\times$ 3 and 2 $\times$ SSC $\times$ 3 all at 42°C for 5 minutes each. Signal detection was achieved using fluorescein isothiocyanate (FITC)-conjugated to avidin and biotinylated antiavidin antibody (Vector Laboratories, Burlingame, CA). Antidigoxigenin rhodamine was added with the first FITC layer to detect chromosome 7 centromeres. Slides were then stained with DAPI and viewed using a Zeiss Axiophot epifluorescence microscope (Carl Zeiss Mikroskop-System, Oberkochen, Germany) with a cooled charge-coupled device (CCD) camera (Astromed, Cambridge, UK) and the IPLabs software supplied by Digital Scientific (Cambridge, UK). At least 15 metaphases per sample/slide were viewed.

*YAC and cosmid clones.* The YAC clones were isolated from a chromosome 7–specific library[18] as were the cosmids; the latter being from the Lawrence Livermore National Laboratory (Berkley, CA). The presence or absence of DNA markers within each genomic clone has been published previously[19,20] or is described for the first time in this study. The insert size of the YAC clones was determined by pulsed-field gel electrophoresis followed by comparison to YPH149 size standards.[21] Specific information on each of the DNA markers is available in the Genome Database (Cambridge, UK).

## RESULTS

*Identification of a family segregating a 7q22.1 inversion breakpoint.* A lymphoblastoid cell line, DD 1027 derived from a patient with a constitutional inversion, 46XY inv7q22.1-34 as the sole cytogenetic abnormality was studied (Fig 2). The patient was phenotypically normal; however, further family cytogenetic analysis showed 10 members of 15 with the same constitutional rearrangement (Fig 3) and 2 cases of hematologic abnormality. An uncle of this patient developed myelodysplasia as a child and has subsequently died after a BMT. An aunt in her early 40s currently has pancytopaenia and BM changes consistent with MDS. Both possessed the inversion. No other members of the family had any evidence of hematologic anomaly and there was no

**Table 1. Hybridization Position of Probes Relative to Breakpoint on Inversion Chromosome 7 on DD1027 Cell Line [46XY inv(7)(q22.1-34)]**

| Probe | Size | Location | DNA Markers Contained in Clone | Hybridization Position |
|---|---|---|---|---|
| Cosmid 4b.1 | | 7q21.1-21.3 | D7S574 | Proximal |
| Cosmid 5.1 | | 7q21.3 | D7S15 | Proximal |
| Cosmid 5.2 | | 7q21.3 | D7S64 | Proximal |
| Cosmid 7b.1 | | 7q21.3 | E1131M30 | Proximal |
| Cosmid 524 | | 7q21.3 | DLX5 | Proximal |
| Cosmid 7c.1 | | 7q22.1 | ASNS | Distal |
| Cosmid 7d.1 | | 7q22 | 932d8.2 | Distal |
| Cosmid 7d.2 | | 7q22 | | Distal |
| Cosmid 8a.1 | | 7q22 | GNB2 | Distal |
| Cosmid 8a.2 | | 7q22 | ACHE | Distal |
| Cosmid 8a.3 | | 7q22 | PLANH1 | Distal |
| Cosmid 8b.1 | | 7q22 | CUTL1 | Distal |
| Cosmid 9.1 | | 7q22 | D7S238 | Distal |
| Cosmid 10.2 | | 7q22-31 | LAMB1 | Distal |
| Cosmid 10.3 | | 7q22-31 | D7S148 | Distal |
| YAC HSC7E800 | 580 kb | 7q21.3-22.1 | DLX5, DLX6 | |
| | | | D7S491, D7S811 | Proximal |
| YAC HSC7E1131 | 1160 kb | 7q21.3-22.1 | DLX5, DLX6 | |
| | | | D7S479, D7S491 | |
| | | | D7S811, D7S821 | |
| | | | AFM088ye9, D7S1796 | Proximal |
| YAC HSC7E783 | 650 kb | 7q21.3-22.1 | ASNS, D7S82 | |
| | | | D7S554 | Across |
| YAC HSC7E1214 | 980 kb | 7q21.3-22.1 | ASNS, TAC2 | |
| | | | D7S82, D7S554 | Distal |

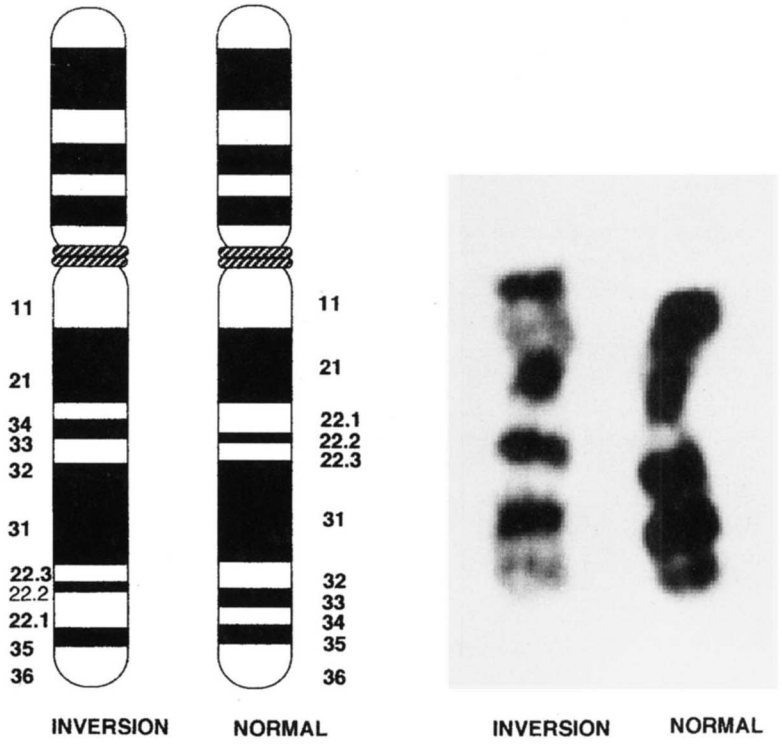



**Fig 2. Idiogram and G-banded images showing normal chromosome 7 and inversion 7q22.1-34.**

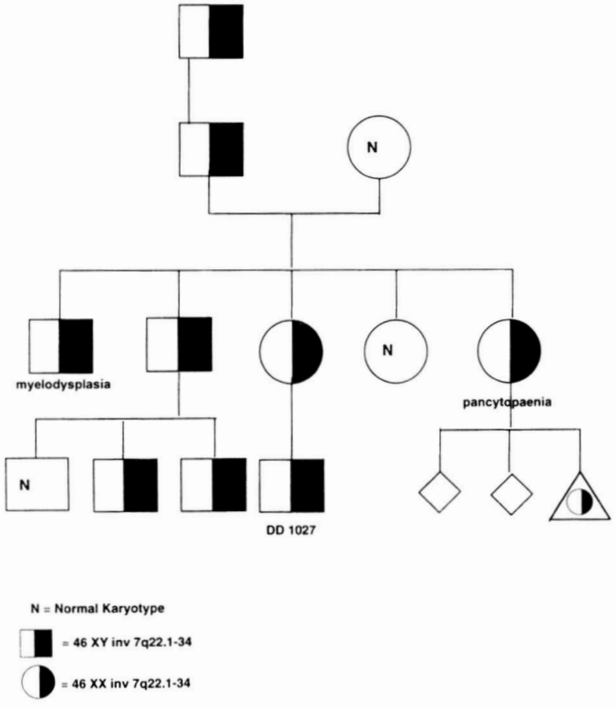



**Fig 3. Family tree to show members affected by constitutional inversion 7q22.1-34.**

history of occupational or environmental exposure to carcinogens.

*Characterisation of the proximal end of a constitutional inversion 7q22.1-q34 associated with MDS.* Fifteen cosmids previously localized and ordered in relationship to each other on band 7q22[22] were hybridized by FISH onto metaphase spreads from the cell line DD 1027 together with a centromeric chromosome 7 probe. The results are summarised in Table 1. The proximal inversion breakpoint was shown to occur between cosmid 524, which is located in its expected position at 7q21.3, and cosmid 7c.1, which hybridizes in the distal position at 7q34 on the inversion chromosome [see (Fig 5a.i) and 5b.i)]. Cosmid 524 has previously been mapped centromeric to cosmid 7c.1[22] and they were the two closest flanking markers available, being separated by approximately 500 kb (Fig 4). Cosmid 7c.1 was identified with an asparagine synthetase (ASNS) gene probe (Table 1).[23]

Four YAC clones (HSC7E800, HSC7E1131, HSC7E1214, HSC7E783) from a contig spanning the region between cosmids 524 and 7c.1[24] (Fig 4) were mapped by FISH against the cell line DD 1027. YAC HSC7E800 and HSC7E1131 were found, as expected, to be centromeric to the proximal breakpoint at 7q22 and HSC7E1214 mapped in the inverted position on the rearranged chromosome. HSC7E783 crossed the breakpoint as split hybridization signals were observed at two positions on the long arm of the inversion chromosome (Fig 5c). All four YACs hybridized to the expected position on the normal chromosome 7 in the DD 1027 cell line and on normal human metaphase chromosomes [(Fig 5 a.ii), b.ii), c.ii)]. A search for genes in this region has been initiated and one previously cloned gene, ASNS, was found to map within HSC7E783.[23]

*Localization of additional breakpoints associated with MDS.* Cells from three additional adult patients with MDS associated with translocations involving 7q22 (Table 2) were examined with YAC HSC7E783. FISH showed a consistent split hybridization signal on both the derivative 2 and 7 chromosomes in cells from patient B and a single hybridization to the normal chromosome 7 and none to the normal chromosome 2 (Fig 6, black and white panels). However, the hybridization of YAC HSC7E783 to the derivative 7 chromosome takes place at 7p. The long arm of this chromosome is also abnormal and more complex than the karyotype reveals. The FISH results suggest a complex rearrangement of the abnormal chromosome 7. The splitting of the YAC signal remains consistent between chromosomes 7 and 2 (with hybridization at the predicted site on the latter) as well as showing signal at 7q22.1 on the normal chromosome 7 demonstrating the single copy specificity of the probe to this region on the long arm. This indicates that in this patient the chromosomal rearrangement involves the same 500-kb region of chromosome 7 as the proximal inversion breakpoint in the DD1027 cell line. The breakpoints in patients A and C were found to be proximal and distal, respec-

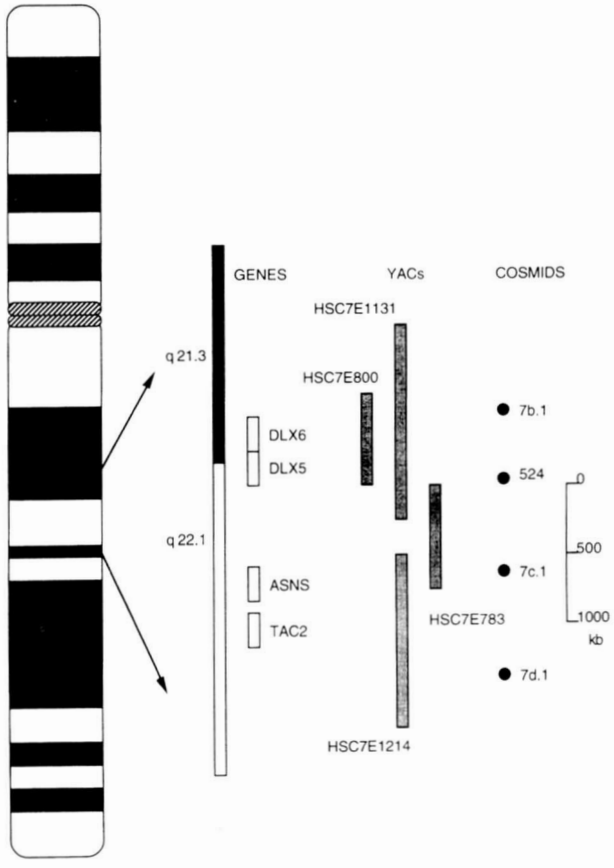



**Fig 4. Position of cosmids, YAC contig, and genes at 7q22.**

**Table 2. Patients With Disease and Karyotype**

| Patient | Phenotype | Karyotype |
|---|---|---|
| A: MD S/1/95 | MDS | 46, XY, t(2;7)(p11;q22) |
| B: MD S/3/95 | MDS | 46, XY, t(2;7)(p11;q21.2) |
| C: MD S/2/95 | MDS | 46, XX, t(2;7)(p11;q22), del(7)(q34q36), der(8) t(8;12)(p21;q11) |
| D: MD S/4/95 | MDS-RARS | 46, XY, del(7)(q22q32) |
| E: AML/5/95 | AML | 46, XY, del(7)(q22q31) |
| F: AML/6/95 | AML | 46-47, XY, −2, −4, del(5)(q23q33) del (7)(q22), +2-3r, +2-4 mar |
| Lymphoblastoid cell line, DD1027 | MDS | 46, XY, inv (7)(q22.1q34) |

tively, to HSC7E783 (Fig 6, patients A and C, Table 2). The exact position of the translocation breakpoints in these patients is currently being determined.

*Deletion in 7q−.* Another three adult patients with 7q22 deletions associated with MDS were identified (Table 2) and BMs examined by FISH with YAC HSC7E783. In all three the hybridization signal was consistently present on the normal, but absent on the abnormal chromosome 7 (Fig 7, Table 2).

## DISCUSSION

The results of this study define a narrow region at 7q22.1 within which a chromosomal rearrangement from two unrelated patients with MDS resides. Examination of patients with MDS and deletions of 7q22 with a YAC clone (HSC7E783) that encompassed the breakpoint region indicated that this interval is also commonly lost in del(7q) patients. These observations are consistent and this region of the chromosome is located in the same general proximal "critical region" as established in other studies (see Fig 1).

However, two of the patients with translocations and MDS show a heterogeneity in the position of their breakpoints although still located within the 7q22 band. Loss of heterozygosity at 7q22 in myeloid disorders, as determined by RFLP studies, has been demonstrated previously.[12] Our findings are in keeping with this data; however, a similar study[13] on five patients suggests there may also be a more distal breakpoint region within 7q22. It is probable that more than one region of chromosome 7q22 contributes to the same type of disease as seen with adults with MDS and 5q−[25] and suggested by the two translocation patients (A and C). It is possible that the breakpoint region identified in our study contains a gene that plays a role in inducing MDS and AML. ASNS has been mapped to YAC HSC7E783.[24] The cos7c.1 probe used in the FISH study was identified using the ASNS cDNA probe and this cosmid was found to be distal to the inversion breakpoint, suggesting that the rearrangement did not directly interrupt this gene. The exact distance of ASNS from the breakpoint has not been determined, but based on the size of HSC7E783 (650 kb) it cannot be more than a few hundred kilobases away.

ASNS is a cell-cycle control enzyme[26] and as such it is being examined as a candidate gene for MDS in these patients. Expression of abnormally low levels of this gene have been reported in AML,[27] further adding to speculation that loss of function of this gene could contribute to the pathogenesis of MDS and AML associated with 7q22.1 rearrangement. A tumor-suppressor gene model could be considered as a mechanism of disease and, if so, it could be postulated that a translocation would render one copy of the gene dysfunctional (either by direct disruption of the gene or through a "position effect"). In the case of monosomy 7 and del (7q), one copy would be lost, whereas the remaining copy of the gene may be inactivated by mutation or methylation.[28,29] This would suggest in the family with the constitutional inversion that such an alteration on the remaining chromosome 7 led to the development of MDS and pancytopenia in the two affected family members. Similar changes to the remaining chromosome 7 in monosomy or 7q- would have the same effect.

A mutation may occur on one allele of one chromosome 7 in a susceptible hematopoietic stem cell, either inherited in familial cases or a somatic mutation. Exposure to mutagenic agents may be responsible for this event in cases of secondary MDS/leukemia. Imprinting has been investigated as a mechanism of disease; however, using RFLP analysis there is currently no firm evidence supporting its existence in familial or nonfamilial cases.[30,31] Gene dosage is another explanation. In this case a critical gene for MDS on chromosome 7 within the deleted region must show haploinsufficiency, ie, two copies of the gene are essential for a normal phenotype. Absolute levels of protein are important in certain developmental pathways and in humans chromosomal aneusomy resulting in hemizygosity for a particular gene may lead to insufficient protein and upset the homeostatic pathway.[9] However, the family described show only a proportion of those with the inversion with the blood disorder, suggesting gene dosage to be an unlikely explanation.

Our study has defined an interval within the q22.1 band of chromosome 7 of importance in MDS. The mapping reagents and data presented here will contribute to ongoing studies to better define regions along 7q critical in MDS. The identification and characterisation of genes (including ASNS) within such critical regions will allow the mechanism of disease to be studied and may suggest alternative approaches to the management of this malignancy.

**Fig 5.** **(see page 3584)** **FISH images: Cosmids 524 and 7cl and YAC HSC7E783 are labeled with biotin and detected with fluorescein giving green signals. The chromosome 7 centromere probe is labeled with digoxigenin and detected with rhodamine giving a red signal. The background staining of the chromosomes is with DAPI. a.i): Cosmid 524 showing hybridization at 7q22 on inversion chromosome 7 and normal chromosome 7 of DD1027 cell line. b.i): cosmid 7cl showing hybridization at 7q22 on normal chromosome 7 of cell line, but distal hybridization on inversion chromosome 7. c.i): YAC HSC7E783 showing split signal on inversion chromosome 7 but expected signal on normal chromosome 7 of cell line. a.ii), b.ii), and c.ii) show hybridization of cosmids 524 and 7cl and YAC HSC7E783 respectively at expected position at 7q22 on both chromosomes 7 on normal metaphase chromosomes.**

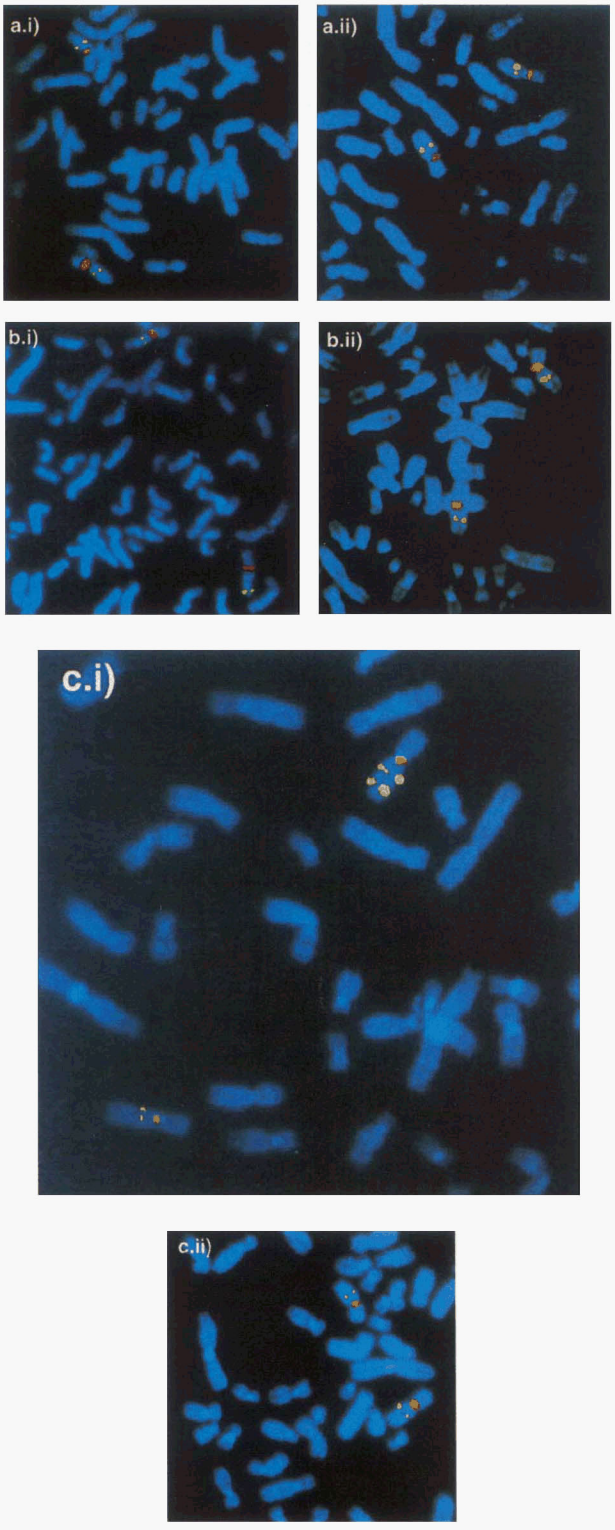



Fig 5.

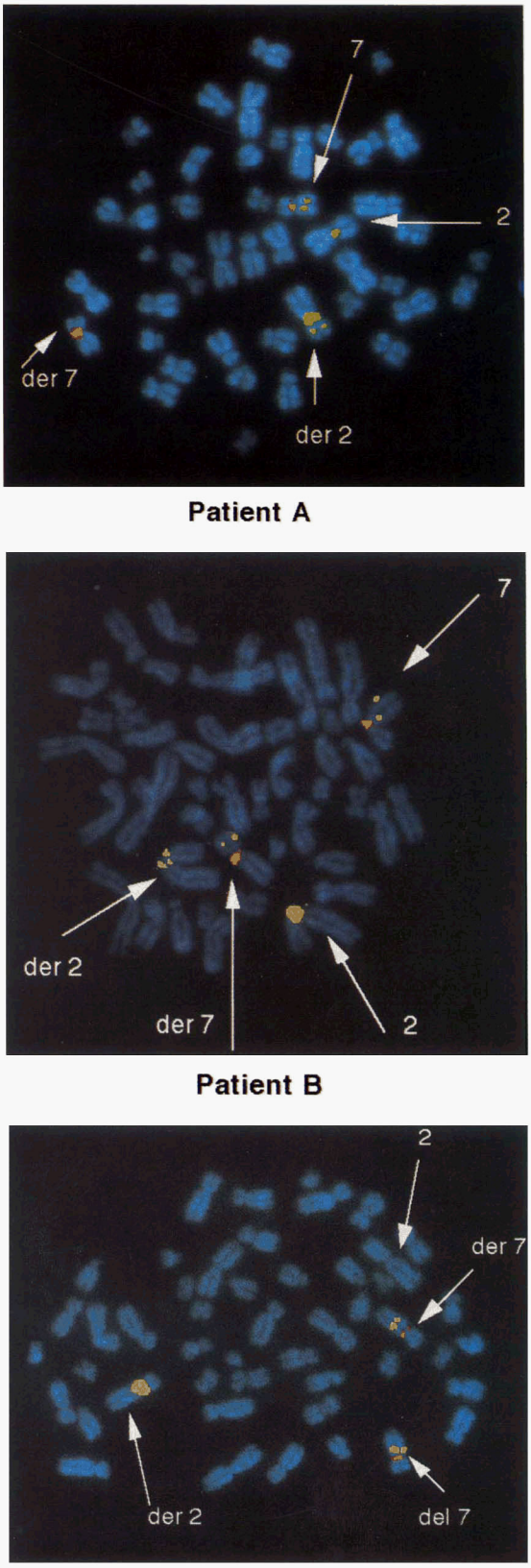
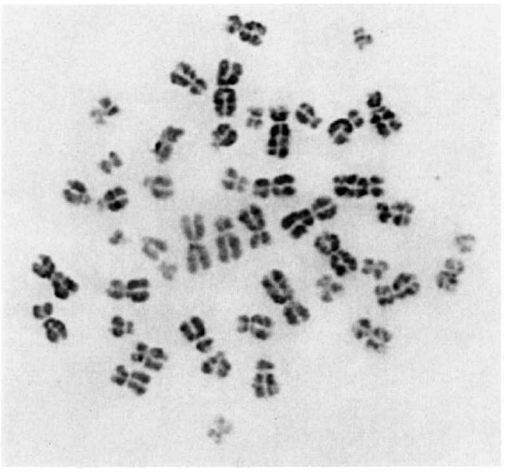
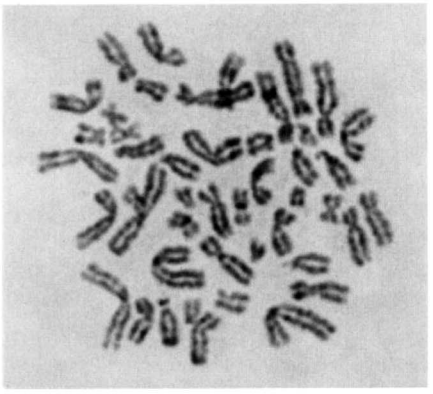


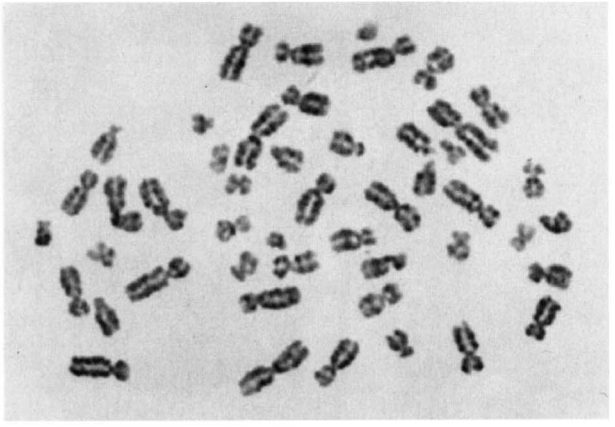

Fig 6.



PATIENT A



PATIENT B



PATIENT C

**Fig 6. (Color panels) YAC HSC7E783 and the chromosome 2 centromere probe are labeled with biotin and detected with fluorescein giving green signals. The chromosome 7 centromere probe is labeled with digoxigenin and detected with rhodamine giving a red signal. The background staining of the chromosomes is with DAPI. Three patients with t(2;7)(p11;q22) each with a different breakpoint as defined by YAC HSC7E783. (Black and white panels) Karyotypes with DAPI banding corresponding to the FISH pictures in the color panels.**

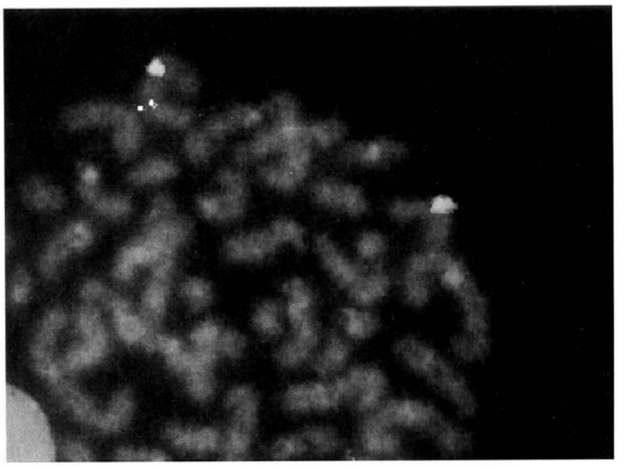


**Fig 7. YAC HSC7E783 is labeled with biotin and detected with fluorescein giving green signals. The chromosome 7 centromere probe is labeled with digoxigenin and detected with rhodamine giving a red signal. The background staining of the chromosomes is with DAPI. Patient with del(7q)- YAC HSC7E783 hybridizes at 7q22 on normal chromosome with loss of signal on deletion chromosome.**

## REFERENCES


1. Sieff CA, Chessells JM, Harvey BAM, Pickthall VJ, Lawler SD: Monosomy 7 in childhood: A myeloproliferative disorder. Br J Haematol 49:235, 1981
2. Tuncer MA, Pagliuca A, Hicsonmez G, Yetgin S, Ozsoylu S, Mufti GJ: Primary myelodysplastic syndrome in children: The clinical experience in 33 cases. Br J Haematol 82:347, 1992
3. Passmore SJ, Hann IM, Stiller CA, Ramani P, Swansbury GJ, Gibbons B, Reeves BR, Chessells JM: Pediatric Myelodysplasia: A study of 68 children and a new prognostic scoring system. Blood 85:1742, 1995
4. Lune-Fineman S, Shannon K, Lange BJ: Childhood monosomy: Epidemiology, biology and mechanistic implications. Blood 85:1985, 1995
5. Neuman WL, Rubin CM, Rios RB, Larson RA, Le Beau MM, Rowley JD, Vardiman JW, Schwartz JL, Farber RA: Chromosomal loss and deletion are the most common mechanisms for loss of heterozygosity from chromosomes 5 and 7 in malignant myeloid disorders. Blood 79:1501, 1992
6. Evans JPM, Czepulkowski B, Gibbons B, Swansbury GJ, Chessells JM: Childhood monosomy 7 revisited. Br J Haematol 69:41, 1988
7. Hansen MF, Cavenee WK: Genetics of cancer predisposition. Cancer Res 47:5518, 1987
8. Johansson B, Mertens F, Mitelman F: Cytogenetic deletion maps of haematologic neoplasms: Circumstantial evidence for tumor suppressor loci. Genes Chromosom Cancer 8:205, 1993
9. Fisher E, Scambler P: Human haploinsufficiency—One for sorrow, two for joy. Nat Genet 7:5, 1994
10. Le Beau M, Albain KS, Larson RA, Vardiman J, Davis E, Blough R, Golomb H, Rowley J: Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: Further evidence for characteristic abnormalities of chromosomes 5 and 7. J Clin Oncol 3:325, 1986
11. Kere J, Ruutu KA, Davies KA, Roninson IB, Watkins PC, Winqvist R, de la Chapelle A: Chromosome 7 long arm deletions

in myeloid disorders: A narrow breakpoint region in 7q22 defined by molecular mapping. Blood 73:230, 1989

12. Kere J, Donis-Keller H, Ruutu T, de la Chapelle A: Chromosome 7 long-arm deletions in myeloid disorders: Terminal DNA sequences are commonly conserved and breakpoints vary. Cytogenet Cell Genet 50:226, 1989

13. Lewis S, Boultwood J, Fidler C, Sheridan H, Said S, Buckle V, Wainscoat JS: Molecular mapping of the 7q deletion in myeloid disorders. Blood 82:34a, 1993 (abstr, suppl 1)

14. Gibbons B, Lillington D, Monard S, Young B, Cheung K, Lister A, Kearney L: Fluorescence in situ hybridisation studies to characterize complete and partial monosomy 7 in myeloid disorders. Genes Chromosom Cancer 10:244, 1994

15. Myers CD, Katz FE, Joshi G, Millar JL: A cell line secreting stimulatory factors for CFU-GEMM culture. Blood 64:152, 1984

16. Ludecke WD, Senger G, Claussen U, Horsthemke B: Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. Nature 338:348, 1989

17. Lengauer C, Green ED, Cremer T: Fluorescence in situ hybridization of YAC clones after Alu-PCR amplification. Genomics 13:826, 1992

18. Scherer SW, Tompkins BJF, Tsui L-C: A human chromosome 7-specific genomic DNA library in yeast artificial chromosomes. Mammal Genome 3:179, 1992

19. Kunz J, Scherer SW, Klawitz I, Soder S, Du Y-Z, Speich N, Kalff-Suske M, Heng H, Tsui LC, Grzeschik K-H: Regional localization of 725 human chromosome 7-specific yeast artificial chromosome (YAC) clones. Genomics 22:439, 1993

20. Scherer SW, Rommens JM, Soder S, Plasvic N, Tompkins BJF, Beattie A, Kim J, Tsui LC: Refined localization and yeast artificial chromosome (YAC) contig-mapping of genes and DNA segments in the 7q21-q32 region. Hum Mol Genet 2:751, 1993

21. Scherer SW, Tsui L-C: Cloning and analysis of large DNA molecules, Adolph K (ed): in Advanced Techniques in Chromosome Research. New York, NY, Dekker, 1991, p 33

22. Scherer SW, Rommens JM, Vandenberg A, Traverso G, Fazil K, Evans JP, Mar L, Osborne L, Tsui L-C: Physical and transcription map of a 25-Mb region on human chromosome 7 (region q21-q22). Cytogenet Cell Genet 71:22, 1995

23. Heng HHQ, Shi X-M, Scherer SW, Andrulis IL, Tsui L-C: Refined localization of the asparagine synthetase gene (ASNS) to chromosome 7, region q21.3 and characterization of the somatic cell hybrid line 4AF/106/KO15. Cytogenet Cell Genet 66:135, 1994

24. Scherer SW, Poorkaj P, Trask B, Soder S, Allen T, Nunez M, Geshuri D, Wong E, Belloni E, Little S, Zhou L, Becker D, Kere J, Ignatius J, Niikawa N, Fukushima Y, Hasegawa T, Weissenbach J, Boncinelli E, Massa H, Tsui L-C, Evans JP: Physical mapping of the split hand/split foot locus (SHFD1) on chromosome 7 and implication in syndromic ectrocactyly. Hum Molec Genet 3:1345, 1994

25. Boultwood J, Lewis S, Wainscoat JS: The 5q- syndrome. Blood 84:3253, 1994

26. Greco A, Ittmann M, Barletta C, Basilico C, Croce CM, Cannizzaro LA, Huebner K: Chromosomal localization of human genes required for G1 progression in mammalian cells. Genomics 4:240, 1989

27. Codegoni AM, Biondi A, Conter V, Masera G, Rambaldi A, D'Incalci M: Human monocytic leukemia expresses low levels of asparagine synthase and is potentially sensitive to l-asparaginase. Leukemia 9:360, 1995

28. Little M, Wainwright B: Methylation and p16: Suppressing the suppressor. Nature Med 1:633, 1995

29. Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC, Baylin SB, Sidransky D: 5' CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. Nature Med 1:686, 1995

30. Shannon KM, Turhan AG, Chang SS, Bowcock AM, Rogers PC, Carroll WL, Cowan MJ, Glader BE, Eaves CJ, Eaves AC, Kan YW: Familial bone marrow monosomy 7. Evidence that the predisposing locus is not on the long arm of chromosome 7. J Clin Invest 84:984, 1989

31. Katz F, Webb D, Gibbons B, Reeves B, McMahon C, Chessells JM, Mitchell C: Possible evidence for genomic imprinting in childhood acute myeloblastic leukaemia associated with monosomy for chromosome 7. Br J Haematol 80:332, 1992

1996 87: 3579-3586

# Molecular definition of a narrow interval at 7q22.1 associated with myelodysplasia

EJ Johnson, SW Scherer, L Osborne, LC Tsui, D Oscier, S Mould and FE Cotter



Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.