1995

A Molecular Cytogenetic Study Of Imprinting In Childhood Acute Lymphoblastic Leukemia (All) Patients With The (1;19) Translocation.

Judith Faye Knops
University of Alabama at Birmingham

A MOLECULAR CYTOGENETIC STUDY OF IMPRINTING IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS WITH THE (1;19) TRANSLOCATION

by

JUDITH FAYE KNOPS

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Program in Medical Genetics in the Graduate School, The University of Alabama at Birmingham

BIRMINGHAM, ALABAMA

1995

ABSTRACT

Genomic imprinting (the functional difference between homologous alleles dependent upon parent of origin) seems to play an important role in some forms of cancer. Cytogenetic polymorphism studies of t(9;22) chromosomes from patients with chronic myelogenous leukemia have shown chromosomes 9 and 22 to be paternal and maternal in origin, respectively. This has suggested a possible imprinting effect on reciprocal translocations in hematological diseases (Haas et al. 1992).

Children with newly diagnosed acute lymphoblastic leukemia (ALL) who were being treated at Pediatric Oncology Group institutions across the United States, had diagnostic bone marrow specimens submitted for cytogenetic analysis. Patients positive for the t(1;19)(q23;p13) were studied to determine the possible involvement of genomic imprinting. The parental origin of the derivative chromosomes 1 and 19 was evaluated using chromosome microdissection and polymerase chain reaction (CMPCR). DNA amplification for the highly polymorphic dinucleotide repeat loci, D1S158, D1S103, D19S49, D19S75 and APOC2, located on the derivative 19, was performed on the microdissected translocated chromosomes. The poor quality and quantity of the archived cytogenetic material required the use of a nested primer approach to increase the sensitivity and specificity of the amplification. In some cases, random pre-amplification of the chromosomal DNA

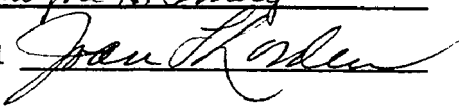
on the microdissected translocated chromosomes. The poor quality and quantity of the archived cytogenetic material required the use of a nested primer approach to increase the sensitivity and specificity of the amplification. In some cases, random pre-amplification of the chromosomal DNA allowed for multiple loci specific amplifications from a single microdissection experiment.

The allele detected on the microdissected chromosomes was compared with parental alleles to assess the parent of origin of the chromosomes involved in the translocation. The ratio of paternal:maternal origin was 4:4 for chromosome 1 and 6:2 for chromosome 19. Evidence for a parental origin bias could not be established with this limited data set.

The existence of parental origin bias is only an indication of a possible imprinting effect, since the bias could result from an unequal activity of the homologous chromosomes. Genomic imprinting may not be involved in the formation of the t(1;19) chromosome found in childhood ALL. The fact that 6 of 8 ALL patients with the t(1;19) have paternal origin of chromosome 19 suggested that additional studies and clinical follow-up were justified to help establish an association between paternal origin of the chromosome 19 and clinical outcome as the disease progressed.

Abstract Approved by: **Committee Chairman** 

Program Director Wayne H. Finley

Date 8/28/95 **Dean of Graduate School** 

ACKNOWLEDGMENTS

I would like to gratefully acknowledge the members of my graduate committee: Dr. Paula Cosper, chairman of my committee, Dr. Andrew Carroll, Drs. Wayne and Sara Finley, Dr. George Brown, and especially Dr. Jian Han. for their constant encouragement and understanding during this investigation and throughout my graduate studies.

I also wish to thank all the parents and patients involved in the study for donating the blood samples needed for the analysis.

Special thanks are due to the entire staff of the Laboratory of Medical Genetics for its assistance during my employment at UAB.

Most of all, I would like to thank all my special friends, who are my family in Birmingham, for their never-ending source of strength and encouragement.

And, last but not least, thanks to my parents, for their loving support in any endeavor I have chosen.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALL	acute lymphoblastic leukemia
AML	acute myelogenous leukemia
bp	base pairs
CpG	cytosine-phosphate-guanine
CML	chronic myelogenous leukemia
CMPCR	chromosome microdissection-PCR
dATP	deoxyadenosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
dCTP	deoxycytidine triphosphate
der	derivative
DNA	deoxyribonucleic acid
GTG	G bands with trypsin and Giemsa
h19	murine H19
H19	human H19
Igf2	murine insulin-like growth factor II gene
IGF2	human insulin-like growth factor II gene
Igf2r	murine insulin-like growth factor II receptor gene
IGF2R	human insulin-like growth factor II receptor gene
kb	kilobases
LOH	loss of heterozygosity
LOI	loss of imprinting
Mash2	murine gene encoding a transcription factor

Ph	Philadelphia chromosome
PCR	polymerase chain reaction
PIC	polymorphic information content
RFLP	restriction fragment length polymorphism
Snrpn	murine small nuclear ribonucleoprotein subunit N
SNRPN	human small nuclear ribonucleoprotein subunit N
TBE	tris HCl, borate, EDTA buffer
UPD	uniparental disomy
Xist	murine gene located at X-inactivation center

INTRODUCTION

Traditional Mendelian inheritance assumes paternally and maternally inherited alleles of a gene are functionally equivalent. The recently described phenomena of genomic imprinting showed this was not always the case (Marx 1988; Monk 1987, 1988; Soltzer 1988; Surani 1986; Hall 1990). Genomic imprinting is a process whereby a specific gene shows a reversible epigenetic modification which often results in differential expression dependent upon parental origin. The nature of the modification is largely unknown but must: 1) involve the gene itself or the chromosomal region in which it resides, 2) occur during gametogenesis or shortly after fertilization, 3) be inheritable by somatic cells, 4) be erased during early germline production in the organism (Rainier and Feinberg 1994). DNA methylation at the CpG sites has appeared to be the principal mechanism for imprinting (Razin and Ceder 1994). The CpG residues can undergo de novo methylation and demethylation at specific sites and the resulting pattern be preserved through several cell generations (Bird 1986; Ceder and Razin 1990). Genomic imprinting has been shown to influence gene expression via a gene dosage mechanism by selectively activating or inactivating an allele from one parent. In recent years, considerable evidence for genomic imprinting has accumulated.

Evidence for Genomic Imprinting

At the whole genome level, studies in mice indicated that both parental complements were needed for normal development (Cattanach and Kirk 1989). The paternal genome appeared to be essential for development of the extra-embryonic tissues and the maternal genome for development of the embryo proper. Zygotes produced from two sperm pronuclei (no maternal contribution) developed normal placentas but lacked embryos; however, those produced from two oocyte pronuclei (no paternal contribution) developed embryological tissue without placentas. In each case, the normal genome content was present but its usual biparental source altered. Similarly in humans, paternally derived diploid conceptions resulted in complete hydatidiform moles (placental tumors), whereas maternally derived diploid conceptions were ovarian teratomas (embryonically derived tumors) (Austin and Hall 1992). Likewise triploid fetuses exhibited different phenotypes dependent on whether the extra set of chromosomes were maternal or paternal in origin (Reik 1989).

At the chromosome level, uniparental chromosomal disomies in which both copies of a chromosome or chromosomal segment were derived from one parent have been observed in all segments of the mouse genome (Searle and Beechey 1978; Cattanach 1986). Systematic experiments have demonstrated that uniparental disomy for chromosomes 2, 6, 7, 11, and 17 were associated with different phenotypes according to parent of origin (Searle et al. 1989). For some chromosomal regions,

paternal disomy resulted in increased growth and maternal disomy in deceased growth suggesting a direct relationship between imprinting and regulation of cell growth (Barton et al. 1991).

In humans, uniparental disomy (UPD), first suggested by Engle (1980) then documented by Spence et al. (1988), has defined possible areas under the influence of genomic imprinting. The most compelling evidence for genomic imprinting in humans through UPD has been the occurrence of two dissimilar disorders, Prader-Willi syndrome (PWS) and Angleman syndrome (AS) with a common chromosome 15 deletion but different parent of origin (Knoll et al. 1989). Although both have been characterized by mental retardation, unusual behavior, and growth disturbance, their phenotypes have been markedly different (Smeets et al. 1992). PWS has been reported in association with the absence of a critical region (15q11-13) of the paternally derived chromosome 15, whereas AS with the deleted maternal chromosome 15. Furthermore, several PWS patients without deletions, were found to have both chromosome 15 homologues derived from the mother (Nicholls et al. 1989).

Another disorder, Beckwith-Wiedemann syndrome (BWS), also may be associated with genomic imprinting, as suggested by paternal uniparental disomy of 11p15 (Viljoen and Ramesar 1992). The BWS has been characterized by multi-organ overgrowth and predisposition to embryonal tumors such as Wilms tumor of the kidney (Pettenati et al. 1986).

Interestingly, the chromosomal segment on 11p, sometimes deleted in BWS, has been found to be homologous to the imprinted mouse chromosome 7, where the insulin-like growth factor II gene (Igf2) and the h19 gene have been mapped.

Evidence for genomic imprinting at the gene level was first derived from studies on transgenic mice. In approximately 20% of the genes inserted into the host genome, expression depended upon the sex of the parent from which it was inherited, irrespective of the position at which they were inserted (Swain et al. 1987; Reik et al. 1987; Surani et al. 1988). Molecular evidence of imprinting has been reported for mouse genes, Igf2, Igf2r, h19, Snrpn, insulin 1 and 2, Xist, and Mash2. Two closely linked imprinted genes, insulin-like growth factor II (Igf2) and h19, have been mapped to chromosome 7. Surprisingly, each was imprinted in the opposite sense, maternally for the Igf2 gene (DeChiara et al. 1991) and paternally in the case of the h19 gene (Bartolomei et al. 1991). A third imprinted gene, insulin-like growth factor II receptor (Igf2r), located in a region of chromosome 17, was found to be transcriptionally inactivated when inherited from the father (Barlow et al. 1991). Leff et al. (1992) found that the Snrpn gene, encoding a protein component from the mRNA splicing machinery, was expressed only by the paternal allele. While both alleles of the two mouse insulin genes were active in embryonic pancreas, only paternal alleles were active in the yolk sac suggesting a tissue and developmental specific imprinting effect (Giddings

et al. 1992). Xist and Mash2 genes have been found to be involved in primary development of the extra-embryonic tissues. Expression of Xist was found to occur first at the 4-cell embryo stage, prior to X-inactivation, only from the paternal allele (Kay et al. 1994). The Mash2 gene, required for trophoblast development, has been recently mapped within the cluster of imprinted genes (Ins2, Igf2 and h19) on distal chromosome 7. The Mash2 paternal allele was found to be initially expressed by groups of trophoblast cells in very early post-implantation stages then completely repressed by 8 days post-coitum (Guillemot et al. 1995).

Analysis of homologous genes and chromosomal areas, as those found imprinted in mouse, has revealed the existence of imprinted genes in humans. The IGF2, H19, and SNRPN genes were found to be imprinted in humans (Giannoukakis et al. 1993; Rainier et al. 1993; Glenn et al. 1993; Surani 1994). Although, the IGF2R gene appeared not to be imprinted (Kalscheur et al. 1993), subsequent data demonstrated exclusive expression from the maternal allele which indicated a functional polymorphism existed in the imprinting of the IGF2R in humans (Xu et al. 1993). Recent evidence has indicated that some genes involved in diabetes might also be imprinted in humans (Haig 1994; Temple et al. 1995)

Imprinting In Human Malignancies

It has been well documented that many genetic abnormalities, including point mutations, chromosome rearrangements, chromosome deletions and amplifications were

associated with cancer. Recently genomic imprinting has been added to this list. By changing chromatin structure and transcription activity, imprinting can be involved in tissue and developmental specific gene regulations (Thomas and Rothstein 1991). Parental-specific gene activation and inactivation provide a mechanism that allows diploid cells to selectively express only one allele of a specific gene. This dosage dependent gene regulation may be disrupted by abnormal imprinting activities which may lead to malignancies. For example, both imprinting (inactivation) of a normally active tumor suppressor gene and loss of imprinting (over-expression) of an oncogene can lead to malignancies.

A major line of evidence for the possible effects of genomic imprinting in malignancies came from the discovery of preferential loss of chromosomes in embryonal tumors (Reik and Surani 1989). These observations raised the possibility that the different function of maternal and paternal alleles caused by genomic imprinting could be a crucial event in the genesis of recessive tumor syndromes. According to Knudson's two hit hypothesis, two mutant alleles encoding a tumor suppressor locus are produced with a mutation of one allele occurring in the germline, followed by the somatic loss of the second functional allele (Knudson 1971). Consequently, the tumor tissues exhibited a loss of heterozygosity (LOH), becoming homozygous for markers located on the chromosome that carried the first mutation. In these heritable cases, the chromosome retained in the tumor is derived from the

affected or carrier parent. However in sporadic cases, most of which are thought to be a consequence of both somatic mutations occurring in a single somatic cell lineage, the maternal and paternal allele would be expected to have an equal chance of carrying the mutation. Investigations have shown this expectation not to be the case. In most sporadic cases of Wilms tumor (Schroeder et al. 1987; Pal et al. 1990), rhabdomyosarcoma (Scrable et al. 1989), retinoblastoma (Dryja 1989; Leach et al. 1990), osteosarcoma (Toguchida et al. 1989), acute myelogenous leukemia (Katz et al. 1992), and neuroblastoma (Caron et al. 1993), the LOH involved a specific parental allele. Except in the case of acute myelogenous leukemia, all of the other cancers mentioned above were associated with a preferential loss of the maternal allele. These observations were consistent with the unequal expression of parental alleles.

The first direct evidence for imprinting in cancer was found in the abnormal expression of IGF2, a growth factor, and H19, a possible tumor suppressor gene, in Wilms tumor (WT). A loss of imprinting (LOI) of the IGF2 gene was described as a possible cause of Wilms tumor (Rainier et al. 1993; Ogawa et al. 1993). In approximately 70% of WT not undergoing LOH, both alleles of IGF2 were expressed; whereas, in normal tissues, only the paternal allele was expressed, indicating a LOI instead. Feinberg (1993) found a difference in methylation of a DNA sequence upstream from the H19 gene between the maternal and paternal alleles in normal tissue.

However, in tumor tissue, the methylation pattern of the maternal copy resembled that of the paternal copy. This methylation pattern switch was accompanied by the activation of the maternal IGF2 allele and the silencing of the maternal H19 which, along with other genetic changes, could cause tumorigenesis (Ezzell 1994). LOI of IGF2 and H19 have been found commonly associated with choriocarcinoma and seemed to be more frequent (50% in native tumor, 75% in tumor cell lines) than in Wilms tumor (Hashimoto et al. 1995).

Additional evidence for imprinting in cancer comes from hereditary cancer syndromes such as hereditary paragangliomas. Pedigree analysis of hereditary paragangliomas (glomus tumors) showed that the clinical manifestation of the disease was determined by the sex of the transmitting parent (van der May et al. 1989). Clinical manifestations of the tumors were exclusively expressed through the paternal line and affected females never transmitted the disease phenotype, even at the subclinical level as detected by magnetic resonance imaging (MRI) (van Gils et al. 1992). This type of transmission pattern strongly suggested genomic imprinting. The responsible gene has been localized to 11q23-qter and the imprinting effect seemed to be absolute (Heutink et al. 1992).

Imprinting in Leukemia

Data has appeared that indicate a possible role of genomic imprinting in leukemia. Katz et al. (1992) found possible evidence for genomic imprinting in childhood acute

myeloblastic leukemia (AML) associated with monosomy 7. Restriction fragment length polymorphism (RFLP) analysis from loci on chromosome 7 of ten patients revealed five out of five patients with myelodysplastic syndrome (MDS) and both patients with de novo AML had loss of paternal alleles, while one patient with monosomy 7 syndrome and two with biphenotypic leukemia had loss of maternal alleles.

Parental Origin Bias in Chronic Myeloid Leukemia

Recently, Haas et al. (1992) reported a possible imprinting effect at the chromosome level. Their work showed that in the translocation leading to the formation of the Philadelphia chromosome (Ph), hallmark of chronic myeloid leukemia (CML), the translocated chromosome 9 was exclusively of paternal descent whereas chromosome 22 was of maternal origin in eleven informative cases. Cytogenetic polymorphisms were used to track the parental origin of the translocated chromosomes. The differently sized centromeric heterochromatin on chromosome 9 and the differently staining nucleolar organizing region on the short arm of 22 were used as markers. Comparison of markers between the parents' chromosomes and the normal and translocated chromosomes in patients allowed for recognition of the parental origin of the normal and the translocated chromosomes 9 and 22.

Translocation at 9q34 and 22q11 has resulted in the reciprocal fusion of the BCR gene (breakpoint cluster region on 22) with the ABL oncogene (homologue of Abelson leukemia

virus on 9). Potential fusion transcripts were produced on the Philadelphia chromosome between the 5′ portion of BCR and the 3′ part of ABL. This fusion gene product has been indicated in leukemic disease and the introduction of the fusion gene alone can cause malignancy (Daley and Ben-Neriah 1991).

If both copies of the chromosomes 9 and 22 had an equal chance for rearrangement, Haas et al. (1992) suggested the observed pattern would result either from a selective expansion of the clone with a particular parent of origin translocation or from certain regions on one homologous chromosome being more susceptible to rearrangement. The latter might be explained by the presence of epigenetic modification such as DNA methylation. Allele specific methylation patterns such as those demonstrated in imprinting (Driscoll et al. 1992; Bartolomei et al. 1993; Stöger et al. 1993; Nicholls 1994a), may cause one copy to be prone to a higher mutation rate.

Subsequent molecular studies in CML (Riggins et al. 1994; Fioretos et al. 1994; Litz and Copenhaver 1994; Melo et al. 1994) demonstrated biallelic expression from both the BCR and ABL genes in peripheral blood indicating these genes were not functionally imprinted in normal leukocytes. However, these studies did not exclude the possibility that abnormal imprinting played a role in the disease-specific selection of the Ph chromosomes, with a specific parent of origin arrangement. However, further molecular studies by Melo et

al. (1995) failed to confirm the cytogenetic data (Haas et al. 1992). Using BCR and ABL specific primers, long-range reverse transcription PCR (RT/PCR) across the BCR/ABL breakpoint was performed. Using an ABL gene polymorphism within the PCR product, they determined in a group of 11 CML patients, that 6 had paternal origin of the ABL gene and 5 had maternal origin.

The most simple explanation postulated for the discrepancy between the cytogenetic and molecular data was an unusually high frequency of yet unproved homologous mitotic recombination events occurring between the centromere and the translocation breakpoints. However, such somatic homologous recombinations have been noted to be extremely rare events and at best could be used to explain a deviation of the nonrandom pattern (Haas 1995). In the cytogenetic study, the patient selection criteria depended upon the presence of informative cytogenetic markers on both chromosomes 9 and 22 (Haas et al. 1992). Likewise, the molecular study was based upon the presence of a BstM restriction fragment length polymorphism in the ABL gene which occurred at a frequency of 8.5% in both normal and leukemic individuals (Melo et al. 1995). Perhaps an ascertainment bias existed for both studies which could have lead to the discordance of results. The question still remains as to whether an imprinting phenomenon can be used to explain the parental origin bias found for the t(9;22) chromosome in CML (Haas 1995; Fioretos et al. 1995; Litz 1995).

Imprinting in Other Leukemia Specific Translocations

Reciprocal translocations in hematological disorders is one of the growing list of mutations possibly influenced by genomic imprinting. The existence of parental origin bias is a strong indication of genomic imprinting, since the bias must result from an unequal activity of the homologous chromosomes. Because translocations originate somatically during the life of the individual, genomic imprinting might be involved either in the formation of the translocation or in its disease-specific selection. If genomic imprinting were involved in the formation of the translocation, only one parental origin combination (i.e., paternal 9 and maternal 22 in CML) should be observed. If genomic imprinting were involved in the disease-specific selection, however, several combinations may occur initially but only the one(s) related with the tumorigenesis will be maintained as a clonal defect. It is possible that more than one parental origin combination is being selected in different patients with the same translocation. In this case, the different parental origin patterns may be responsible for the clinical heterogeneity.

The Possibility of Genomic Imprinting in Childhood ALL Patients with the t(1;19)(q23;p13)

The t(1;19)(q23;p13) chromosome has been found to be the most common translocation in childhood acute lymphoblastic leukemia, with an overall incidence of 5%-6% (Carroll et al.

1984; Raimondi 1993). It has been closely associated with the pre-B immunophenotype. Clinically, the presence of the t(1;19) has been correlated significantly with several recognized prognostic features such as a positive immunophenotype for cytoplasmic Ig, higher leukocyte counts, and higher serum lactate dehydrogenase levels (Pui et al. 1990). As expected, the t(1;19) is associated with an inferior outcome in patients treated on minimally or moderately intensified protocols (Crist et al. 1990). This rearrangement occurred in either a balanced, t(1;19)(q23;p13),or unbalanced, -19, +der(19)t(1;19)(q23;p13) form. In the unbalanced form, two normal chromosomes 1 were always present and probably represented a duplication of the normal homologue. Some leukemic cells contained both forms, implying that the der(1) could be lost through clonal evolution, without loss of the transformed phenotype.

As in the t(9;22) in CML, the t(1;19)in ALL has been studied at the molecular level. The translocation has been reported to produce a critical fusion of the 5' end of E2A and the 3' end of the PBX1 genes (Mellentin et al., 1989; Kamps et al. 1990; Nourse et al., 1990). E2A which encodes the Ig enhancer binding factors E12 and E47, has been mapped to 19p13, while PBX1, a homeobox gene of unknown function, to 1q23. The genomic breakpoints appeared to cluster in a constant junction site resulting in the chimeric E2A-PBX1 transcript (Hunger et al. 1991). The hybrid E2A-PBX1 gene product contributed to leukemic cell growth (Kamps et al.

1991), most likely through altering the transcriptional properties of the PBX1 homeodomain protein (Lu, et al. 1994; LeBrun and Cleary 1994).

The chromosome 19 in the t(1;19) has regional homology to mouse chromosome 7 which has been shown to be imprinted (Searle et al, 1989). Translocations involved in this region may result in the juxtaposition of two differently imprinted domains, thus exerting different 'position effects' on the adjacent genes depending on the domains involved. Altered expression of these genes might be involved in particular clinical and biological features of the disease such as the cIg^+ immunophenotype and poor prognosis. Therefore, a parental origin bias of the t(1;19) would be a strong indication of a genomic imprinting effect in childhood ALL.

Methods for Parental Origin Studies

Use of cytogenetic polymorphisms has been one technique for determining the parent of origin, but few chromosomes exhibit informative polymorphisms and the analysis can sometimes be erroneous. Because balanced translocations do not cause a deletion or duplication of DNA, traditional molecular approaches are not readily amenable for determining parent of origin in most hematological diseases. Most molecular techniques would be feasible only in those cases with intragenic polymorphisms which would be altered by the rearrangement. Long range PCR could be used if the sequence of the genes involved in the translocation were known and informative polymorphisms could be identified in close

proximity to the breakpoints. Alternatively, the homologous chromosomes involved in the translocation need to be separated by either generating somatic cell hybrids (Kruse and Patterson 1973) or by chromosome microdissection before analysis for molecular polymorphisms (Knops et al. 1993).

Chromosome microdissection polymerase chain reaction (CMPCR) with analysis of molecular polymorphisms such as microsatellite repeats has provided the most direct and simple approach for imprinting studies of leukemia specific chromosome translocations. Since the first report by Lüdecke et al. (1989), a variety of chromosome microdissection techniques have emerged (Kao 1990; Senger et al. 1990; Kao and Yu 1991; Hadano et al. 1991; Hirota et al. 1992; Meltzer et al. 1992). Many of these techniques involved micromanipulation of the chromosome fragments into nanoliter droplets for extraction, restriction enzyme digest and linker ligation to a primer sequence which proved to be very tedious. The chromosome microdissection PCR (CMPCR) technique has allowed direct PCR amplification in prepared buffer of specific DNA fragments from a minimal number of copies (Han et al. 1991). CMPCR has been successfully used to map specific band locations for several genes (Han et al. 1991; Eipers et al. 1991; Lu et al. 1992, George et al. 1993; George et al. 1995) from a single chromosome fragment.

Simple sequence tandem repeats known as microsatellites have provided the best source of DNA polymorphisms because they were found to be universally distributed in the human

genome, to demonstrate a high degree of polymorphism, and to be easily typed (Weber and May 1989). Microsatellites have been found to consist of 10-50 copies of motifs from 1-6 base pairs that can occur in perfect tandem repetition, as imperfect repeats or together with another repeat type. Dinucleotide CA repeats were the most common and occurred about every 30 kb (Hudson et al 1992). A large portion of microsatellites have four or more alleles and a polymorphic information content (PIC) greater than 0.70, making them ideal genetic markers (Hearne et al. 1992). These repeats have been amplified and analyzed from single sperm cells (Hubert et al. 1992) as well as from microdissected chromosomes (Spielvogel et al. 1992).

CMPCR in association with molecular analysis using microsatellite repeats has provided a powerful new technique to investigate cytogenetic abnormalities at the molecular level. To determine if genomic imprinting occurred in other hematological malignancies, the CMPCR technique was used to investigate parental origin of recurring chromosome rearrangements in the rare pre-B cell childhood acute lymphoblastic leukemia (ALL). One of the more frequently recurring rearrangements in this subgroup of childhood ALL, the t(1;19)(q23;p13), was studied by comparing the polymorphisms found in microdissected translocated chromosomes from cytogenetic preparations from bone marrow of the patient with those found in the genomic DNA of their parents. Information from these studies could provide an

approach to understanding the role of imprinting in this subgroup of childhood ALL.

MATERIALS AND METHODS

Patient Material

Children with newly diagnosed acute lymphoblastic leukemia (ALL) who were being treated at Pediatric Oncology Group institutions across the United States, had diagnostic bone marrow specimens submitted to the Laboratory of Medical Genetics at University of Alabama at Birmingham for cytogenetic analysis. Patients positive for the t(1;19)(q23;p13) (Figure 1), with or without other chromosomal abnormalities, were studied. Table 1 lists the karyotype interpretation of the nine patients involved in the study.

Preparation of Metaphase Spreads from Bone Marrow Specimens

Cell Culture

The GTG-banded metaphase chromosomes from bone marrow were prepared by standard cytogenetic techniques (Barch 1991). Using sterile technique, duplicate cultures were initiated using 10 ml. RPMI 1640 (Gibco) supplemented with 15% fetal bovine serum (Gibco), 1% L-glutamine (200 mM) (Gibco), and ~0.3 ml bone marrow aspirate. Cultures were incubated overnight at 37°C.

Cell Harvest

After the incubation period, the cultures were treated with 0.05 ml Colcimid (10 µg/ml) (Gibco) for 2.5 hours at

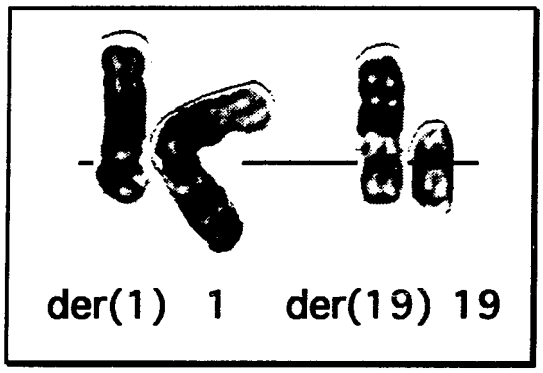


Figure 1. GTG banded chromosomes with the t(1;19)(q23;p13).

Table 1

Cytogenetic Diagnosis of the Patients with Childhood ALL.

PATIENT	ABNORMAL KARYOTYPE[a]
01JV	46,XY,-19,+der(19)t(1;19)(q23;p13)
04RC	46,XX,t(1;19)(q23;p13)
07PH	47,XY,t(1;19)(q23;p13),+i(1q)
10JM	47,XY,t(1;19)(q23;p13),del(13q),+21
13YM	46,XX,-19,+der(19)t(1;19)(q23;p13)
16HS	46,XY,-19,+der(19)t(1;19)(q23;p13)
19ME	46,XX,t(1;19)(q23;p13),t(13q14q)c
22CC	46,XX,t(1;19)(q23;p13)
25AC	46,XY,-19,+der(19)t(1;19)(q23;p13)

[a] This diagnosis includes only the predominant abnormal cell line. The complete cytogenetic diagnosis can be found in the appendix.

4°C. The cell culture suspensions were transferred to 15 ml conical centrifuge tubes and centrifuged at 1500 rpm for 6 minutes. The supernatant was discarded and the cell pellet resuspended. Twelve ml 0.075 KCl hypotonic solution was added. After 20 minutes, the hypotonic action was stopped with addition of 2 ml of 3:1 methanol:acetic acid fixative. The fixative/hypotonic cell suspension was mixed and after 15 minutes, centrifuged at 1500 rpm for 6 minutes. The supernatant was discarded, the cell pellet resuspended, and 12 ml 3:1 methanol:acetic acid fixative added. After 10 minutes, the cell suspension was centrifuged at 1500 rpm for 10 minutes. The cell pellet was washed three times with 4-5 ml fresh 3:1 methanol:acetic acid fixative.

Slide Preparation and Banding

After appropriate dilution in fresh fixative, several drops of cell suspension were dropped onto clean wet microscope slides, and metaphases spread by placing the slide on a 56°C slide warmer to dry. The slides were aged at 90°C for 30 minutes. GTG bands were obtained by pretreatment of slides in 0.5 % trypsin/Hank's balanced salt solution without Ca^{++} and Mg^{++} (HBSS) (Gibco) for 5-10 seconds then rinsed twice in HBSS. The slides were stained using 4% Giemsa (Harleco) in pH 6.8 Gurr's phosphate buffer (Gibco) for 3 minutes, then rinsed in distilled water and air dried. The stained cytogenetic preparations were stored in moist chambers until needed.

Preparation of Genomic DNA

Patient and parental blood was requested from the referring physicians. Most patients had both parents available for study, but in some cases only maternal blood was obtained. Approximately 10 ml of venous blood was collected in EDTA vacutainers and shipped overnight. Upon receipt, the blood was frozen at -20°C until needed for extraction of DNA.

DNA Extraction

DNA extraction was performed using a routine phenol-chloroform procedure (Sambrook et al. 1989). The frozen blood was thawed, transferred to a 50 ml Corex centrifuge tube and mixed with an equal volume of phosphate buffered saline (Gibco). The blood/PBS solution was centrifuged using a Beckman model J 21B ultracentrifuge at 3500g for 15 minute. The supernatant was discarded and the cell pellet resuspended. Fifteen ml DNA extraction buffer (10 mM Tris-HCl (pH 8.0); 0.1M EDTA (pH 8.0); 20 µg/ml pancreatic RNase A; 0.5% SDS) was added. Proteinase K (Sigma) was added to a final concentration of 100 mg/ml. The mixture was incubated with gentle agitation at 37°C.

After overnight incubation, three extractions with phenol were performed by adding equal volumes of Tris equilibrated phenol (pH 8.0) (USB), gently mixing and separating by centrifugation at 5000g for 15 minutes. The organic phase was removed and discarded and the aqueous phase phenol extracted two more times. After the third phenol

extraction, equal volumes of 24:1 chloroform:isoamyl alcohol was added to the aqueous phase, mixed, and centrifuged at 5000g for 15 minutes. The aqueous phase was transferred to a clean 50 ml plastic conical centrifuge tube.

The DNA was precipitated by adding 0.2 volumes of 10M ammonium acetate and 2 volumes of 100% cold ethanol. Using a glass hook, the DNA was transferred and resuspended in 500 µl sterile water. The DNA was quantified using a Milton Roy 1201 spectrophotometer. The DNA was stored at -20°C until needed.

Microsatellite Repeat Loci and Primers

Microsatellite Repeat Loci

Microsatellite repeat loci were selected from those in which primers were commercially available as Mappairs™ (Research Genetics). Those loci located near the breakpoint on the derivative 19 with the highest degree of polymorphism available were used (Tables 2 and 3). The polymorphic information content (PIC) provided a useful index of informativeness for a marker. The PIC was calculated from allele frequencies in the population and was related to the mean repeat length (Hearne et al. 1992).

Primer Sets

A set of primers external to the published set of primers for each microsatellite repeat loci were designed using the OLIGO program. A nested primer approach, where two rounds of PCR amplification were performed, was used to increase the specificity of amplification from limited

Table 2

Microsatellite Repeat Loci for Chromosome 1.

Locus	Chromosomal Location	PIC[a]	Reference
D1S158	1q32-q41	0.89	Overbeck et al. (1992)
D1S103	1q32-qter	0.80	Weber et al. (1990c)

[a] Polymorphic information content.

Table 3

Microsatellite Repeat Loci for Chromosome 19.

Locus	Chromosomal Location	PIC[a]	Reference
D19S49	19q12-q13.1	0.74	Weber et al. (1990b)
D19S75	19q12-q13.1	0.64	Weber et al. (1990a)
APOC2	19q13.1	0.85	Weber and May (1989)

[a] Polymorphic information content.

numbers of starting templates when working with microdissected chromosome fragments. Fully nested primer sets for D1S158, D1S103, D19S49, and D19S75 were designed (Figure 2) while hemi-nested primers were used for APOC2 (Figure 3). Primer sequences and product size for external (primer 1 and 2) and internal (primer 3 and 4) are found in Table 4.

Polymerase Chain Reaction (PCR)

Optimal PCR conditions were obtained on genomic DNA in a series of reactions varying the magnesium chloride concentration from 1.0 mM to 3.0 mM and the temperature from 45°C to 65°C. The reactions were performed in a final volume of 25µl of 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl,

0.1 mg/ml gelatin, 0.2 mM each dATP, dGTP, dTTP, dCTP buffer with 100 ng genomic DNA, 1.0 ng/µl final concentration of primers and 2U Taq polymerase (Boehringer Mannheim). Thirty PCR cycles of 94°C denaturation for 1 minute, optimal annealing temperature for 2 minutes, and 72°C extension for 2 minutes was performed, with a final extension of 72°C for 8 minutes using a MJResearch Mini Cycler. The product was detected by electrophoresing in a 1% agarose gel with ethidium bromide in Tris-Borate-EDTA buffer(TBE).

Allele detection reactions were performed on genomic DNA from the patients and their parents to determine which loci were informative. The reactions were performed in a final volume of 25 µl 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, 0.2 mM each dATP, dGTP, dTTP, dCTP buffer with 100 ng genomic DNA, 1.0 ng/µl final concentration

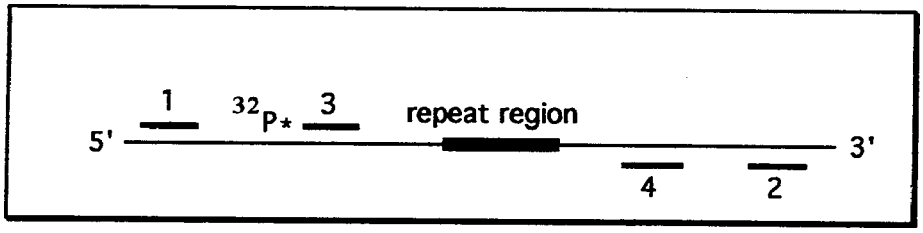


Figure 2. Fully nested PCR primers for the amplification of dinucleotide repeat loci. Primers 1 and 2 were used for the first round amplification, and 3 and 4 were used in the second round reaction for detection. Primer 3 was end-labeled with γ ^{32}P-ATP.

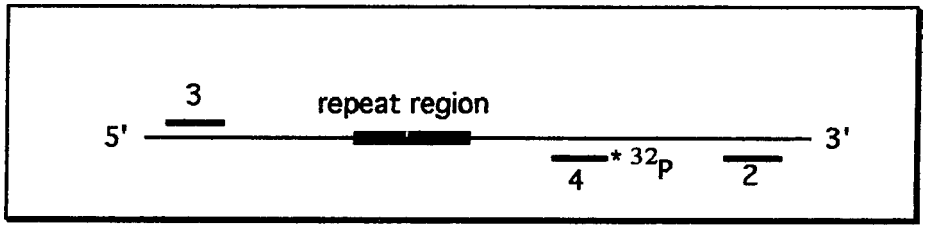


Figure 3. Hemi-nested PCR primers for the amplification of dinucleotide repeat loci. Primers 2 and 3 were used for the first round amplification, and 3 and 4 were used in the second round reaction for detection. Primer 4 was end-labeled with γ ^{32}P-ATP.

Table 4

Primer Sequences and Average Product Size for Dinucleotide Repeat Loci.

PRIMER SET	SEQUENCE	SIZE[a]
D1S158 external[b]	5' TCTTTGTTCTTTGTTTCTTG 3'	340
	5' TCAAAGAAGAGAAGATAACC 3'	
D1S158 internal[c]	5' GGGCCTTCTTATATTGCTTC 3'	150
	5' GGAAAGACTGGACCAAAGAG 3'	
D1S103 external[b]	5' ATCTCTCAGCTATTACAAGG 3'	203
	5' TGACAGTGATTTTAGTTGGT 3'	
D1S103 internal[c]	5' ACGAACATTCTACAAGTTAC 3'	85
	5' TTTCAGAGAAACTGACCTGT 3'	
D19S49 external[b]	5' GCTCTCGGACCATAACAAGT 3'	220
	5' TCACCTCAGCCTCCCAAAGT 3'	
D19S49 internal[c]	5' ACTCATGAAGGTGACAGTTC 3'	120
	5' GTGTTGTTGACCTATTGCAT 3'	
D19S75 external[b]	5' ATCAGCTTCCCTTTGCTCCC 3'	240
	5' ATCTGTTATTTTAGCAAGCA 3'	
D19S75 internal[c]	5' ATTATTCCATCTAAAAGCGAA 3'	143
	5' TTCCCTTTGCTCCCCAAACG 3'	
APOC2 external[d]	5' AGCCCGTGTTGGAACCATGA 3'	202
	5' GTGATTTGTGGAGTGTGGTG 3'	
APOC2 internal[d]	5' GCTTGAGCCCAGGAGTTTGA 3'	136

[a] product size is listed in base pairs (bp), [b] the external primer sets were used for the first round of amplification, [c] the internal primer sets were used for the second round of amplification, [d] locus APOC2 was amplified using a single internal primer in conjunction with one external primer.

internal primers 3 and 4 and 2U Taq polymerase (Boehringer Mannheim). Primer 3 (100 ng) was end-labeled in 20 µl 70 mM Tris-HCl (pH 7.6); 10 mM $MgCl_2$; 5 mM DTT with 66µM γ-^{32}P-ATP (6000 Ci/mmol) (Amersham) using T4 polynucleotide kinase (New England Biolabs) at 37°C for 1 hour. Thirty PCR cycles of 94°C denaturation for 1 minute; optimum annealing temperature for 2 minutes, and 72°C extension for 2 minutes was performed, with a final extension of 72°C for 8 minutes using a MJResearch Mini Cycler.

Polyacrylamide Gel Electrophoresis

The products were electrophoresed on a 60 cm IBI BaseRunner 100 (Kodak) using a 6% sequencing gel (Sequegel-6™, National Diagnostics) in TBE buffer (USB) at 50 W for 2.5-4 hours. A ϕX174/Hindf marker was γ ^{32}P end-labeled in 20 µl 70 mM Tris-HCl (pH 7.6); 10 mM $MgCl_2$; 5 mM DTT with 66 µM γ ^{32}P-ATP (6000 Ci/mmol) (Amersham) using T4 polynucleotide kinase (New England Biolabs) at 37°C for 1 hour and electrophoresed with the products as a size control. The gel was transferred to blotting paper and exposed to X-ray film (Kodak) for 4-24 hours depending upon the intensity of the signal.

Chromosome Microdissection

Microdissection 6f the bone marrow chromosomes was performed on a Olympus BH2 light microscope using a long working distance objective. The slides were scanned under 250X magnification and appropriate metaphases chosen for microdissection. Metaphases were examined and the

translocation chromosomes identified under 1000X magnification. Chromosome microdissection was then performed under 625X magnification.

Chromosome microdissection was accomplished with borosilicate glass needles pulled to a tip diameter of 0.5 µm or less using a Kopf Model 2D pipette puller. The micromanipulator (Narishige) was used to position the glass needles below the chromosome. Immediately after positioning, the needle was driven upward along the chromosome axis from one terminus to the other to scrape the chromosome off the surface of the slide. Then, the needle was immediately lifted with the chromosome adhering to the tip (Figure 4). Usually 2 to 10 chromosomes were microdissected individually and each chromosome carefully transferred by immersion of the needle tip directly into 25 µl sterile PCR buffer.

The der(19) was microdissected for PCR analysis of microsatellite repeat loci in all patients studied. In the event the translocation was balanced, the normal 1 and/or 19 were microdissected. Similarly, the normal 19 was microdissected if the translocation was unbalanced.

PCR from Microdissected Chromosomes

The first round of amplification was performed in 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, 0.2 mM each dATP, dGTP, dTTP, dCTP buffer containing 0.1 ng/µl of external primers (1 and 2) specific for one of the five microsatellite repeat loci (D1S158, D1S103, D19S49,

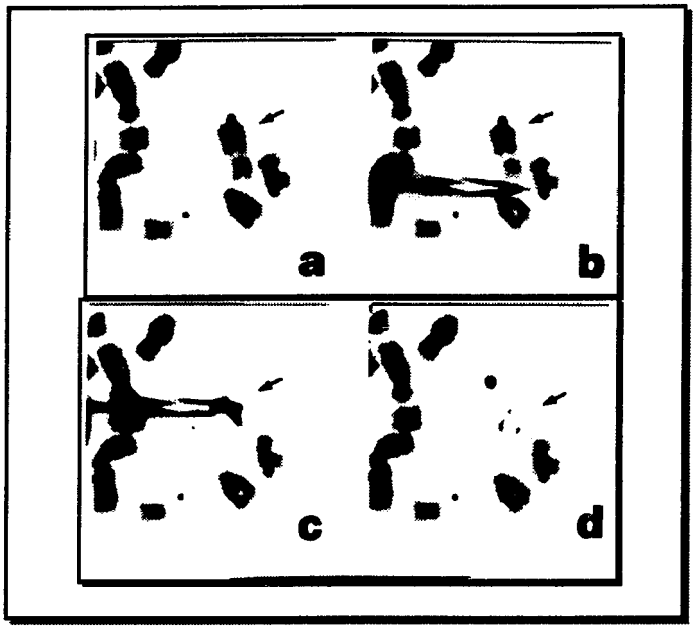


Figure 4. Example of chromosome microdissection of der(19) from a bone marrow metaphase spread. The arrow indicates the der (19) to be cut. a) der(19) before dissection, b) needle in position for cutting, c) partially cut chromosome, d) after cutting, chromosome has been transferred into PCR buffer.

D19S75, APOC2). After 10 minutes at 100°C, 2 U Taq polymerase (Boehringer Mannheim) was added and 5 PCR cycles of 94°C denaturation for 1 minute, a programmed ramp of +4°C over 4 minutes, and 72°C extension for 2 minutes were performed. The ramping step began at 47°C for D1S158, 51°C for D1S103, 52°C for D19S49 and D19S75, and 56°C for APOC2. Then, 45 PCR cycles of 94°C denaturation for 1 minute, 52°C annealing for D1S158, 54°C annealing for D1S103, 55°C annealing for D19S49 and D19S75, and 60°C annealing for APOC2, then 3 minutes 72°C extension for 2 minutes was performed.

A second round amplification was performed using internal primers 3 and 4 (primer 3 was 5' end-labeled with γ ^{32}P). Twenty-five µl PCR buffer (as above) with 0.2 ng/µl internal primers was sterilely transferred into the first round reaction tube. Fifty PCR cycles of 94°C denaturation for 1 minute, 55°C annealing for 2 minute, and 72°C extension for 3 minutes were performed.

Amplification Using Nonspecific Primers

Alternatively, the microdissected chromosomes were preamplified using one of several nonspecific primers. At lower annealing temperatures, nonspecific primers annealled at random sites along the original template (the microdissected chromosomes), and subsequent extension increased the original copy number of the target DNA. Three random amplification techniques, R-1, R-2, and R-3, used different primers with specific conditions.

R-1 Technique

In this technique, multiple copies of the chromosomal DNA were produced with fifty rounds of primer extension reactions using a collection of 15-base oligonucleotides in which any one of the four bases could be present in any position (Zhang et al. 1992). The chromosomes were cut into an alkaline buffer (200 mM KOH; 50 mM dithiothreitol) then neutralized with an equal volume of 900 mM Tris-HCl, pH 8.3, 300 mM KCl, and 200 mM HCl. Fifty µl K+ free PCR buffer (2.5 mM $MgCl_2$; 10 mM Tris-HCl, pH 8.3; 0.1 mg/ml gelatin) with 0.2 mM each dNTP, 50 ng/µl random primer and 5 U Taq polymerase (Perkin-Elmer/Cetus) was added. Fifty PCR cycles of 94°C denaturation for 1 minute; 37°C annealing for 2 minutes, then a programmed ramp of 10 second/degree to 55°C, and extension at 55°C for 4 minutes were performed.

R-2 Technique

In this technique, the universal oligonucleotide 5'-CCGACTCGANNNNNNNATGTGG-3' was used to produce multiple copies of the original chromosome after relaxation of the DNA with topoisomerase I (Guan et al. 1993). The reaction buffer (40 mM Tris-HCl, pH 7.5; 20 mM $MgCl_2$, 50 mM NaCl; 0.2 mM each dNTP; 36 ng/µl universal primer) was first purified with a Micron 30 (Amicon). The chromosomes were transferred into 5 µl of purified buffer and one unit of topoisomerase I (Promega) added before incubation at 37°C for 30 minutes. An initial eight cycles of primer extensions were performed

using T7 polymerase (Sequenase, Version 2.0, USB) which functioned better at lower temperatures than Taq polymerase. The chromosomal DNA was denatured at 94°C for 1 minute, then 0.3 U T7 polymerase (Sequenase Version 2.0, USB) was added, followed by primer annealing at 30°C for 2 minutes and extension at 37°C for 2 minutes. After this initial primer extension, conventional PCR was performed in the same reaction tube. Fifty µl PCR reaction mixture (10 mM Tris-HCl, pH 8.3; 1.5 mM $MgCl_2$, 50 mM KCl; 0.1 mg/ml gelatin, 0.2 mM each dNTP and 2 U Taq polymerase (Perkin-Elmer/Cetus) was added. After 3 minutes at 95°C, 40-50 PCR cycles of 94°C denaturation for 1 minute, 56°C annealing for 2 minute, and 72°C extension for 3 minutes were performed.

R-3 Technique

A variation of the previous procedure also was used to randomly amplify the chromosomal DNA by first annealing the universal primer A, 5'-TGGTAGCTCTGATCANNNNN-3' at low temperature and extending with T7 polymerase then targeting the 5' end of the universal primer A with 5'-AGAGTTGGTAGCTCTTGATC-3' (primer B) during the conventional PCR cycling (Bohlander et al. 1992). The microdissected chromosomes were transferred into 5µl buffer A containing 40 mM Tris-HCl, pH 7.5; 2.0 mM $MgCl_2$, 50 mM NaCl; 0.2 mM each dNTP, and 50 ng/µl primer A. After denaturation at 96°C and cooling to 4°C for primer annealing, one unit of Taq polymerase (Boehringer Mannheim) was added in 2.5 µl of

buffer A and the temperature ramped to 37°C over an 8 minute interval then held at 37°C for 8 minutes. After a second round of low temperature amplification, conventional PCR was carried out by adding 90 µl of buffer B (10 mM Tris-HCl, pH 8.3; 1.5 mM $MgCl_2$, 50 mM KCl; 0.1 mg/ml gelatin, 0.2 mM each dNTP, 50 ng/µl primer B) to the same reaction tube. Five low stringency PCR cycles with denaturation at 94°C for 1 minute, annealing at 42°C for 5 minutes, a ramp to 72°C over 6 minutes, and extension at 72°C for 5 minutes were followed by 33 PCR cycles of 94°C denaturation for 1 minute, 56°C annealing for 1 minute and, 74°C extension for 2 minutes.

Loci Specific Amplification After Preamplification

After preamplification of chromosomal DNA, a small aliquot of the reaction mixture was removed for amplification with the nested loci specific primers as described above. If loci specific product was not obtained the preamplified products from all the reactions were combined, the oil removed with equal volumes of chloroform, and then precipitated using 0.1 volumes of 10 N ammonium acetate and 2 volumes of cold 100% ethanol. The DNA was resuspended in a small volume of sterile water (usually 2-5µl). The concentrated preamplified products were again used for amplification with the nested loci specific primers.

All PCR reactions were performed with at least one negative control containing all reaction reagents except template (genomic DNA, microdissected chromosomal DNA or

preamplified chromosomal DNA). The negative control from the preamplification procedure was also used as template for amplification with the nested loci specific primers. Generally, positive controls using genomic DNA were not done because of the possibility for cross contamination of genomic DNA with microdissected chromosomal DNA. If a signal similar to the chromosomal DNA experimental reaction was detected in the negative control, the results were not used.

Polyacrylamide Gel Electrophoresis Analysis of Chromosomal DNA

The PCR reaction mixture (3-4 µl) was electrophoresed on a 30 cm IBI BaseRunner 100 (Kodak) using a 6% sequencing gel (Sequegel-6™, National Diagnostics) in TBE buffer (USB) at 35W for 1 hour to determine if a product amplified from the chromosomal DNA was present. A γ ^{32}P end-labeled ϕX174/Hindf marker was electrophoresed with the products as a size control. The gel was transferred to blotting paper and exposed to X-ray film (Kodak) for 4-24 hours, depending on the intensity of the signal.

If a signal was present within the proper size range for a particular loci with no similar signal in negative controls, the product was electrophoresed beside the detection reaction products from patient and parental genomic DNA. The allele amplified from the microdissected chromosome was compared to the two alleles detected in the patient and then traced back to the original parental allele to determine the parent of origin for that chromosome.

RESULTS

Determining Optimum PCR Conditions for Nested Primer Sets

The primer sets were used with various magnesium concentrations, annealing temperatures and Taq polymerases to amplify small quantities of genomic DNA in order to obtain optimal PCR conditions. For most primers, optimal magnesium concentration was 1.5 mM using Boehringer Mannheim Taq polymerase. Perkin-Elmer Cetus Taq polymerase along with PerfectMatch® (Promega) was utilized to obtain optimal results for the APOC2 locus with 1.5 mM $MgCl_2$. The optimal annealing temperatures for each primer set are listed in Table 5.

Two primer sets, D1S158 and D19S49, proved to be very effective in achieving a distinct product without significant background amplification. Occasionally, additional rounds of amplification (10-15) were needed to produce adequate product using primer sets for the remaining loci (D1S103, D19S75, and APOC2).

Numerous attempts to establish consistent amplification from chromosomal DNA microdissected from standard leukocyte cytogenetic preparations were unsuccessful. Leukocyte cytogenetic preparations were used because of the better quality and quantity of metaphases than those from bone marrow cytogenetic preparations. Nevertheless, amplification

Table 5

Optimum Annealing Temperatures for the Nested Sets of Primers used in the Analysis of the t(1;19) Chromosome.

Loci	External	Internal
D1S158[a].....	52°C	55°C
D1S103[a].....	54°C	56°C
D19S49[b].....	55°C	55°C
D19S75[b].....	55°C	56°C
APOC2[b].....	60°C	62°C

[a] Located on chromosome 1 [b] Located on chromosome 19.

was achieved sporadically at best. Therefore, attempts to achieve consistency were abandoned. Microdissected chromosomal DNA from patient bone marrow preparations were amplified using conditions optimized on genomic DNA with results occurring irregularly.

Determination of Informative Loci

The dinucleotide repeat loci were considered informative if the 2 alleles of the patient were heterozygous and parents did not share the same set of alleles. Informative loci for chromosomes 1 and 19 were ascertained by analysis of genomic DNA from the patient and both parents. For patient 13YM and 25AC only maternal blood was available. At least one informative loci for each chromosome was detected for all families involved in the study. Table 6 shows the informative status of the families for the five loci used in the study.

Table 6

Informative Status for Microsatellite Repeat Loci from Chromosomes 1 and 19 in All Families Studied.

	Chromosome 1		Chromosome 19		
Patient	D1S158	D1S103	D19S49	APOC2	D19S75
01JV.....	INF	INF	INF	INF	INF
04RC.....	INF	NI	NI	INF	INF
07PH.....	INF	INF	NI	INF	UND
10JM.....	INF	INF	INF	NI	NI
13YM.....	INF	INF	INF	NI	UND
16HS.....	INF	UND	INF	UND	UND
19ME.....	INF	NI	INF	INF	UND
22CC.....	INF	NI	NI	UND	INF
25AC.....	INF	UND	INF	UND	NI

INF: informative; NI: not informative; UND: undetermined

For the two most effective loci, D1S158 was informative for all families studied while D19S49 was informative for ~70%. D1S103, APOC2, and D19S75 were undetermined for some families because the analysis was successfully completed with other loci.

Analysis of D19S49 and D1S158 from 01JV and 04RC are shown in Figure 5 and 6. D19S49 was highly informative for 01JV but uninformative for 04RC who was homozygous for the parental upper allele. D1S158 was highly informative for 04RC with several base pairs separating the two patient alleles. Although informative, the two alleles for 01JV were only 2

Figure 5. Informative status of patients 01JV and 04RC for locus D19S49 located on chromosome 19. Lanes 1, 2, and 3, Patient 01JV, his mother and father, respectively. Lanes 4, 5, and 6, Patient 04RC, her mother and father, respectively. As the results indicate, 01JV was informative for the D19S49 locus, since he inherited allele 1 from his mother and allele 4 from his father. 04RC, on the other hand, was not informative since she inherited allele 2 from both her mother and father.

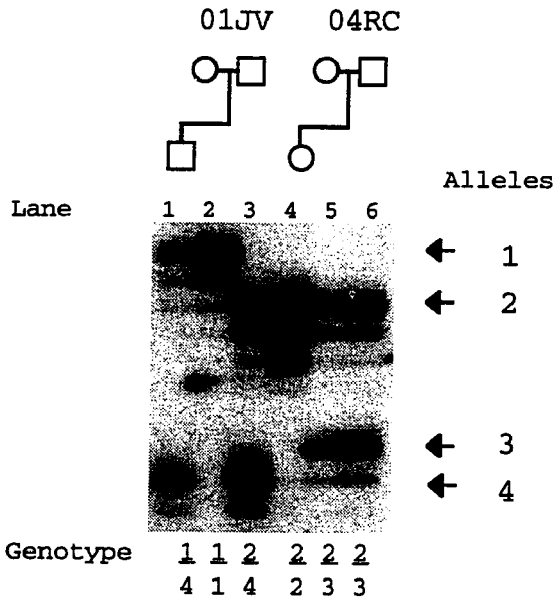
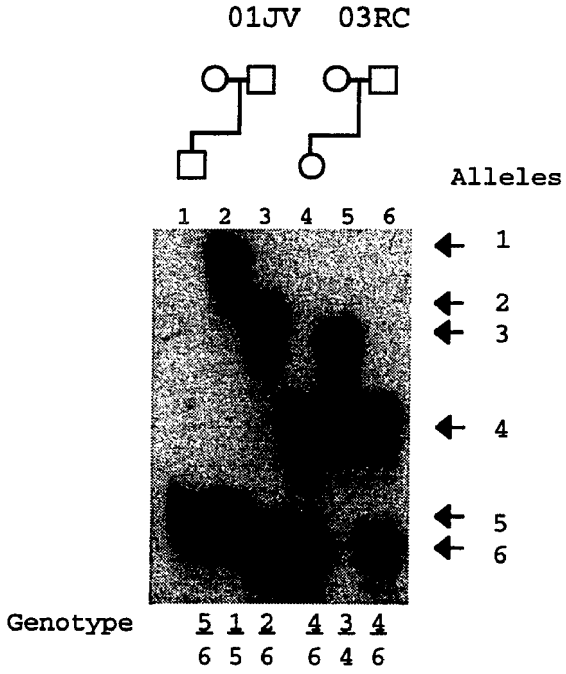

01JV
04RC
Alleles
Lane
1 2 3 4 5 6
1
2
3
4
Genotype
1 1 2 2 2 2
4 1 4 2 3 3

Figure 6. Informative status of patients 01JV and 04RC for locus D1S158 located on chromosome 1. Lanes 1,2, and 3, Patient 01JV, his mother and father, respectively. Lanes 4, 5, and 6, Patient 04RC, her mother and father, respectively. As the results indicated, 01JV, was informative for the D1S158 locus, although the alleles he inherited (allele 5 from his mother and allele 6 from his father) had minimal separation (2 base pairs). On the other hand, the D1S158 locus for 04RC, was more useful since alleles 4 and 6 had a higher degree of separation.


01JV 03RC
Alleles
1 2 3 4 5 6
1
2
3
4
5
6
Genotype
5 1 2 4 3 4
6 5 6 6 4 6

base pairs different and therefore, not as useful. Highly informative loci were detected in all families except 19ME (chromosome 1), 04RC (chromosome 19), 25AC (chromosome 19),and 07PH (chromosome 19) where the informative loci used for analysis had a 2 to 4 base pair difference between the patient's alleles.

Results of Amplification of Microsatellite Repeat Loci from Chromosomal DNA

Amplification of microsatellite repeat loci from chromosomal DNA was achieved sporadically. Numerous attempts on all patient samples were performed using both direct amplification of repeat loci and preamplification (using random primers) prior to amplification of repeat loci. Table 7 shows those microsatellite repeat loci successfully amplified from microdissected chromosomal DNA. Since the translocation is clonal in origin and would involve a single translocation event, only one allele was amplified from the microdissected chromosomes. The products generated were used for parental origin analysis.

In patients with unbalanced karyotypes (01JV, 13YM, 16HS, and 25AC), the normal homologue for chromosome 1 was not microdissected and amplified (ND). If results were obtained from the der(19), then the normal homologues were not microdissected and amplified (ND), or were classified as unsuccessful because attempts to amplify were discontinued (UNS). Amplification from patient 22CC was unsuccessful due to limited amount of chromosomal DNA available for analysis.

Table 7

Microsatellite Repeat Loci Successfully Amplified from Chromosomal DNA.

	Chromosome 1		Chromosome 19	
Patient	der(19)	normal 1	der(19)	normal 19
01JV.....	D1S103[b]	ND	D19S49	D19S49
04RC.....	D1S158	D1S103	APOC2[a]	UNS
07PH.....	D1S103[c]	ND	APOC2[c]	ND
10JM.....	D1S158[b]	ND	D19S49[b]	ND
13YM.....	D1S158	ND	D19S49[b]	UNS
16HS.....	D1S158[a]	ND	D19S49[b]	UNS
19ME.....	D1S158	UNS	D19S49	UNS
22CC.....	UNS	UNS	UNS	UNS
25AC.....	D1S158	ND	D19S49	D19S49

ND:not done; UNS:unsuccessful amplification, [a] from pre-amplified product using R-1 technique, [b] from pre-amplified product using R- 2 technique, [c] combination of the two.

Four previously banded slides were received from the referring clinical laboratory but did not yield sufficient numbers of metaphases for the numerous attempts required to obtain results.

In one case, 10JM, the chromosome morphology was so poor the normal homologues of the translocation were mostly unidentifiable; therefore, only the der(19) was microdissected and amplified.

Direct Amplification from Chromosomal DNA

Of the 19 total loci specific amplifications, ten (53%) were successful using the microdissected chromosomal DNA directly as the template. The microdissected der(19) chromosomes from four patients (04RC, 13YM, 19ME, and 25AC) were directly amplified using D1S158 primer sets, and from three patients (01JV, 19ME, and 25AC) using D19S49 primer sets. In addition, the normal 1 homologue from 04RC and the normal 19 homologues from 01JV and 25AC were directly amplified using D1S103 and D19S49 primer sets.

Preamplification Using Nonspecific Primers

Preamplified chromosomal DNA products served as a template for almost half (9/19) of the loci successfully amplified. The products generated from the R-2 technique produced results for 5 loci, D1S103 on 01JV, D1S158 and D19S49 on 10JM, D19S49 on 13YM, and D19S49 on 16HS. Amplification from D1S158 on 16HS and APOC2 on 04RC were from products generated by a collection of random 15-base oligonucleotides as primers (R-1 technique). Amplification from D1S103 and APOC2 on 07PH resulted from combining preamplified chromosomal DNA products from all three techniques(R-1, R-2, and R-3).

In general, amplification across dinucleotide repeats from microdissected chromosomal DNA was inconsistent, most likely due to the nature of DNA subjected to cytogenetic procedures such as acid fixation of the cells. Fortunately, results were obtained with persistence and numerous attempts.

Direct amplification from microdissected chromosomal DNA performed best for those loci that initially gave a strong signal on genomic DNA (i.e., D1S158 and D19S49). Preamplification of the microdissected chromosomal DNA increased the original template copy number providing a better possibility for successful amplification from some primers (i.e., D1S103). Preamplification also allowed for amplification of different loci from the same microdissected chromosome at various times as was done for patient 07PH. A preamplified product was used to obtain results first from D1S103. After optimizing conditions for the APOC2 primers, the original preamplified product was used to obtain results from the APOC2 locus.

Analysis of Parental Origin of the t(1;19) Chromosome

Upon successful amplification of a specific locus, the product was electrophoresed next to detection reaction products from patient and parental genomic DNA. By comparing the allelic status with those amplified from genomic DNA, the parental origin of the translocated chromosome was determined.

Parental origin analysis of the t(1;19) for patient 01JV indicated chromosome 1 was maternal in origin while chromosome 19 was paternal in origin (Figure 7). The paternal genotype for locus D1S103 was 1/4, and the maternal was 2/3. Patient 01JV inherited the maternal 2 allele and the paternal 4 allele. The allele amplified from the microdissected der(19) was within the size range of the patient's maternally

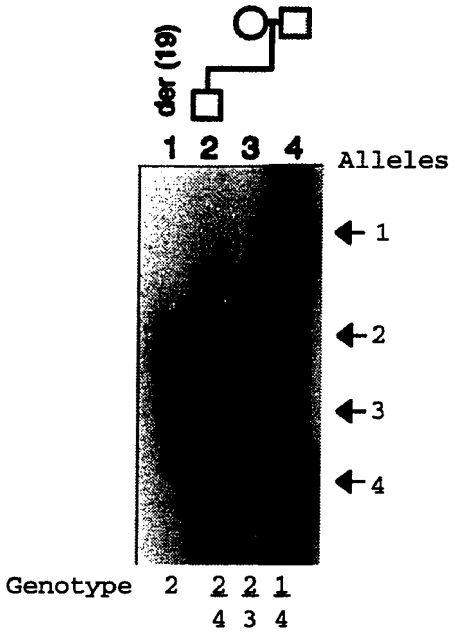
inherited 2 allele. Therefore, the chromosome 1 involved in the translocation was maternal in origin. Likewise, for locus D19S49 (Figure 8), the paternal genotype was 2/4, the maternal genotype 1/3 and the patient genotype 1/4, with allele 1 maternally derived and allele 4 paternally derived. The allele amplified from the der(19) was the paternal 4 allele indicating paternal origin for chromosome 19. The normal 19 from 01JV was also analyzed using locus D19S49 (Figure 9). As expected, the maternal 1 allele was amplified from the normal 19 homologue confirming the paternal origin of the 19 involved in the translocation.

Autoradiographs showing parental origin of the der(19) in the remaining 7 patients are shown in Figures 10 through 25. As with patient 01JV, the allele detected in the microdissected der(19) was compared with the patient alleles to determine parental origin of chromosomes 1 and 19. For patient 04RC the normal 1 homologue (Figure 12) and for patient 25AC the normal 19 homologue (Figure 25) were analyzed and confirmatory results found.

The "stutter" or "shadow" bands normally seen from slipped-strand pairing were a problem when using nested primers across dinucleotide repeats. Because the final PCR product was so dependent upon the first few cycles of the reaction, stutter bands amplified in the secondary amplification produced stronger signals as seen in Figure 12. Therefore, highly informative loci with the patient alleles separated by more than 2 repeats were best for analysis.

Figure 7. Maternal origin of the chromosome 1 involved in the t(1;19) for patient 01JV. The autoradiograph from D1S103 showed allele 2 detected on the der(19) corresponded to the maternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 01JV. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

Figure 8. Paternal origin of the chromosome 19 involved in the t(1;19) for patient 01JV. The autoradiograph from D19S49 showed allele 3 detected on the der(19) corresponded to the paternally derived allele Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 01JV. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.


der (19)
1 2 3 4
Alleles
1
2
3
4
Genotype 2 2 4 2 3 1 4

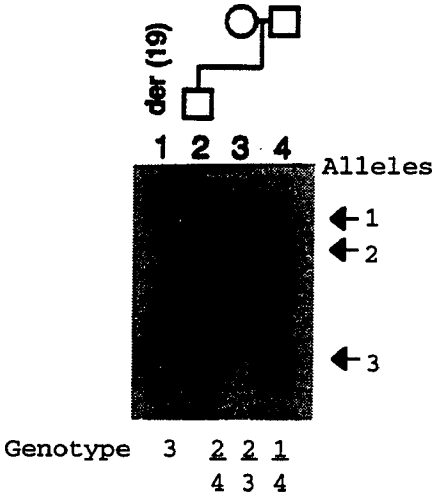

der (19)
1 2 3 4
Alleles
1
2
3
Genotype 3 2 4 2 3 1 4

Figure 9. Maternal origin of the normal chromosome 19 homologue for patient 01JV. The autoradiograph from D19S49 showed allele 1 detected on the normal 19 corresponded to the maternally derived allele. This result confirmed the paternal origin of the chromosome 19 involved in the t(1;19).Lane 1, Microdissected chromosome 19. Lane 2, Genomic DNA from patient 01JV. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

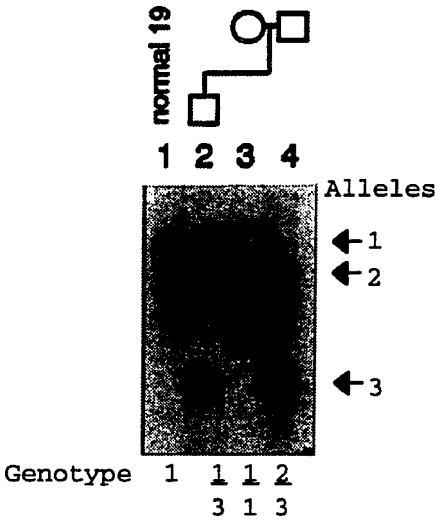
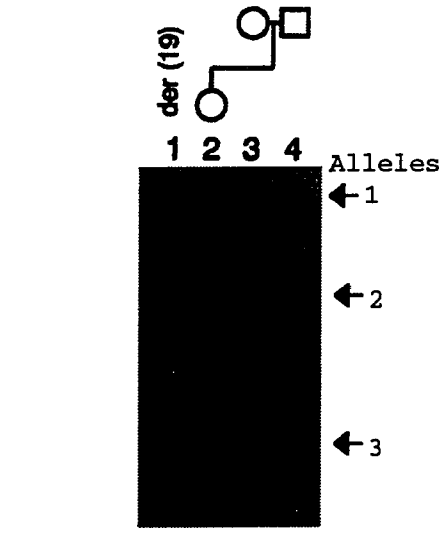
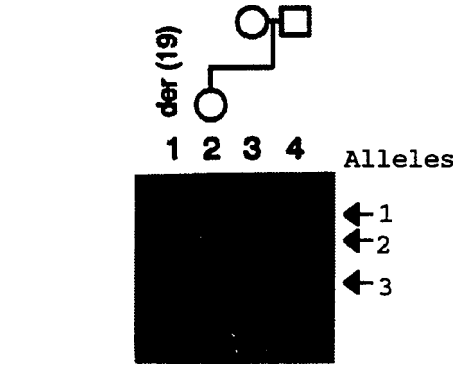

normal 19
1 2 3 4
Alleles
1
2
3
Genotype 1 1 1 2
3 1 3

Figure 10. Maternal origin of the chromosome 1 involved in the t(1;19) for patient 04RC. The autoradiograph from D1S158 showed allele 2 detected on the der(19) corresponded to the maternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 04RC. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

Figure 11. Paternal origin of the chromosome 19 involved in the t(1;19) for patient 04RC. The autoradiograph from APOC2 showed allele 1 detected on the der(19) corresponded to the paternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 04RC. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.



Genotype 2 $\frac{2}{3}$ $\frac{1}{2}$ $\frac{2}{3}$



Genotype 1 $\frac{1}{3}$ $\frac{3}{3}$ $\frac{1}{2}$

Figure 12. Paternal origin of the normal chromosome 1 homologue for patient 04RC. The autoradiograph from D1S103 showed allele 1 detected on the normal chromosome 1 corresponded to the paternally derived allele. This result confirmed the maternal origin of the chromosome 1 involved in the t(1;19). Lane 1, Microdissected chromosome 1. Lane 2, Genomic DNA from patient 04RC. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

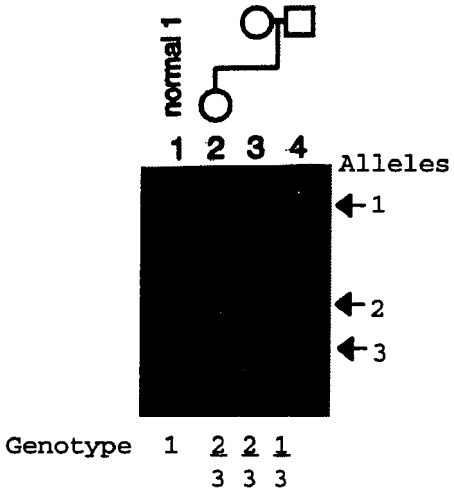
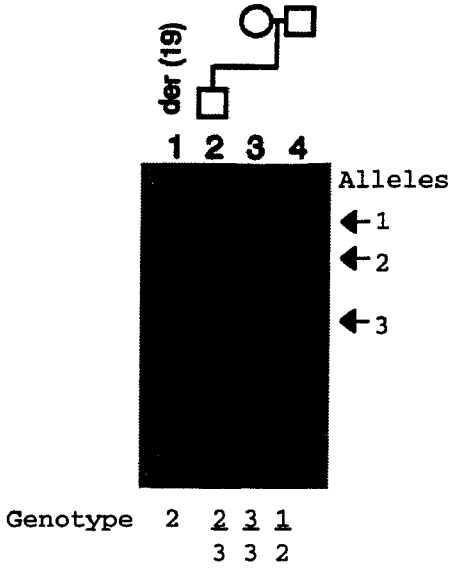

normal 1
1 2 3 4
Alleles
1
2
3
Genotype 1 2 2 1
3 3 3

Figure 13. Paternal origin of the chromosome 1 involved in the t(1;19) for patient 07PH. The autoradiograph from D1S103 showed allele 2 detected on the der(19) corresponded to the paternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 07PH. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

Figure 14. Paternal origin of the chromosome 19 involved in the t(1;19) for patient 07PH. The autoradiograph from APOC2 showed allele 2 detected on the der(19) corresponded to the paternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 07PH. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.


der (19)
1 2 3 4
Alleles
1
2
3
Genotype 2 2 3 1
3 3 2

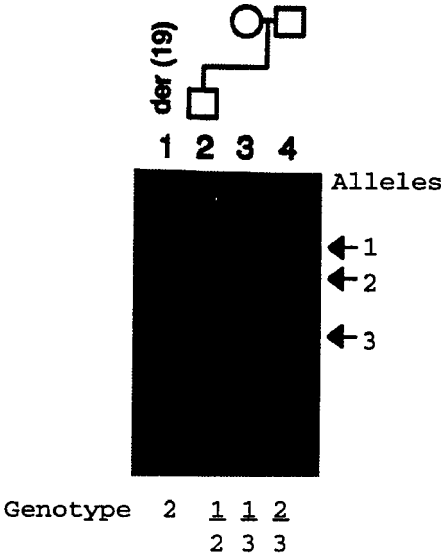
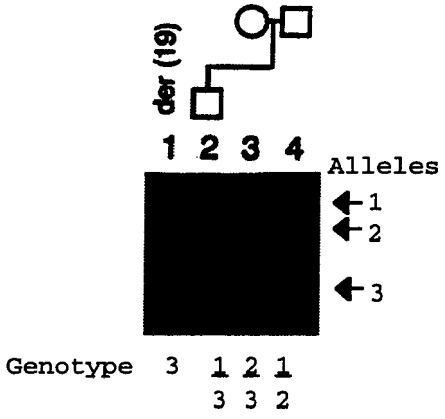

der (19)
1 2 3 4
Alleles
1
2
3
Genotype 2 1 1 2
2 3 3

Figure 15. Maternal origin of the chromosome 1 involved in the t(1;19) for patient 10JM. The autoradiograph from D1S158 showed allele 3 detected on the der(19) corresponded to the maternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 10JM. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

Figure 16. Maternal origin of the chromosome 19 involved in the t(1;19) for patient 10JM. The autoradiograph from D19S49 showed allele 2 detected on the der(19) corresponded to the maternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 10JM. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.


der (19)
1 2 3 4
Alleles
1
2
3
Genotype 3 1 2 1
3 3 2

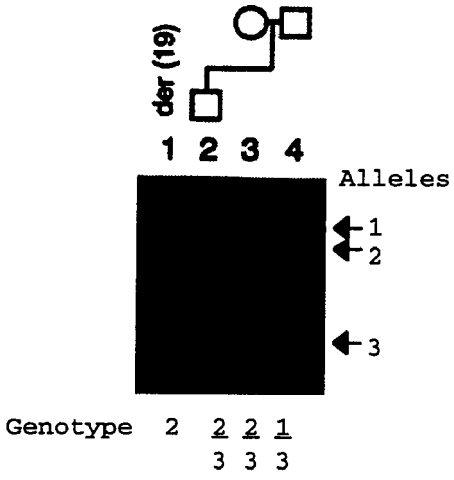
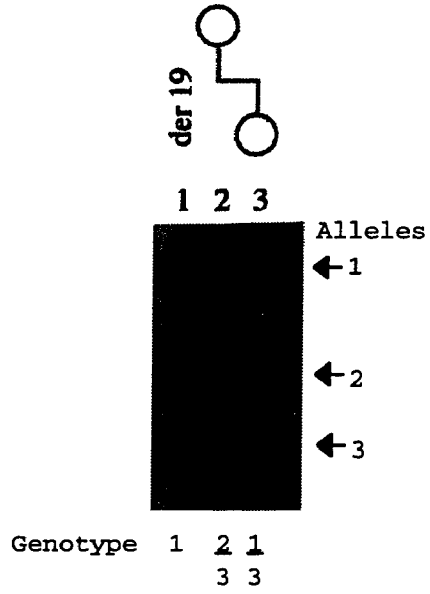

der (19)
1 2 3 4
Alleles
1
2
3
Genotype 2 2 2 1
3 3 3

Figure 17. Paternal origin of the chromosome 1 involved in the t(1;19) for patient 13YM. The autoradiograph from D1S158 showed allele 1 detected on the der(19) corresponded to the paternally derived allele. Because the paternal genomic DNA was unavailable, the patient's allele 1 was assumed to be paternally derived since allele 3 was maternally derived. Lane 1, Microdissected chromosome der(19). Lane 2, Maternal genomic DNA. Lane 3, Genomic DNA from patient 13YM.

Figure 18. Paternal origin of the chromosome 19 involved in the t(1;19) for patient 13YM. The autoradiograph from D19S49 showed allele 1 detected on the der(19) corresponded to the paternally derived allele. Because the paternal genomic DNA was unavailable, the patient's allele 1 was assumed to be paternally derived since allele 3 was maternally derived. Lane 1, Microdissected chromosome der(19). Lane 2, Maternal genomic DNA. Lane 3, genomic DNA from patient 13YM.


der 19
1 2 3
Alleles
1
2
3
Genotype 1 2 1
3 3

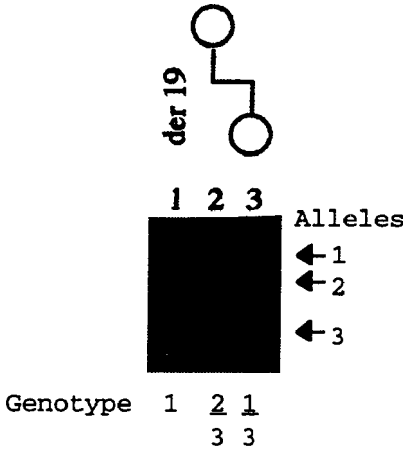
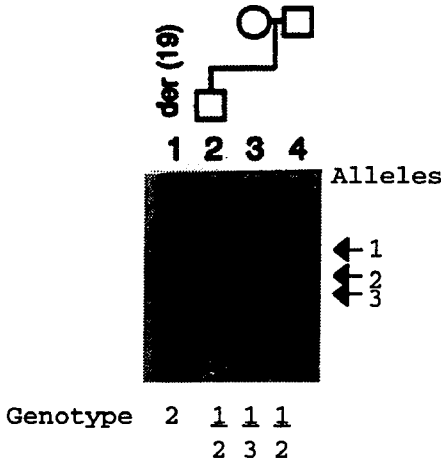

der 19
1 2 3
Alleles
1
2
3
Genotype 1 2 1
3 3

Figure 19. Paternal origin of the chromosome 1 involved in the t(1;19) for patient 16HS. The autoradiograph from D1S103 showed allele 2 detected on the der(19) corresponded to the paternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 16HS. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

Figure 20. Paternal origin of the chromosome 19 involved in the t(1;19) for patient 16HS. The autoradiograph from D19S49 showed allele 3 detected on the der(19) corresponded to the paternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 16HS. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.


der (19)
1 2 3 4
Alleles
1
2
3
Genotype 2 1 1 1
2 3 2

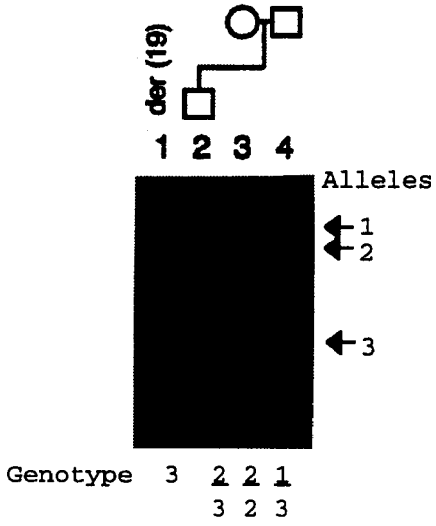
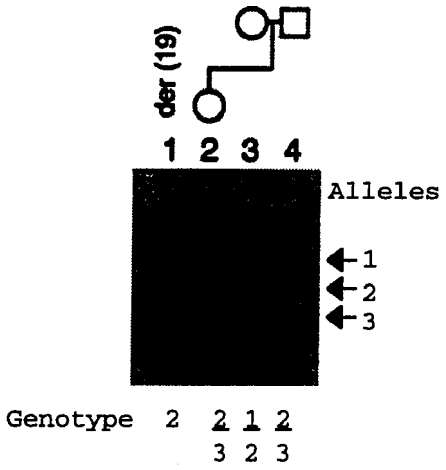

der (19)
1 2 3 4
Alleles
1
2
3
Genotype 3 2 2 1
3 2 3

Figure 21. Maternal origin of the chromosome 1 involved in the t(1;19) for patient 19ME. The autoradiograph from D1S158 showed allele 2 detected on the der(19) corresponded to the maternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 19ME. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.

Figure 22. Paternal origin of the chromosome 19 involved in the t(1;19) for patient 19ME. The autoradiograph from D19S49 showed allele 2 detected on the der(19) corresponded to the paternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 19ME. Lane 3, Maternal genomic DNA. Lane 4, Paternal genomic DNA.


der (19)
1 2 3 4
Alleles
1
2
3
Genotype 2 2 1 2
3 2 3

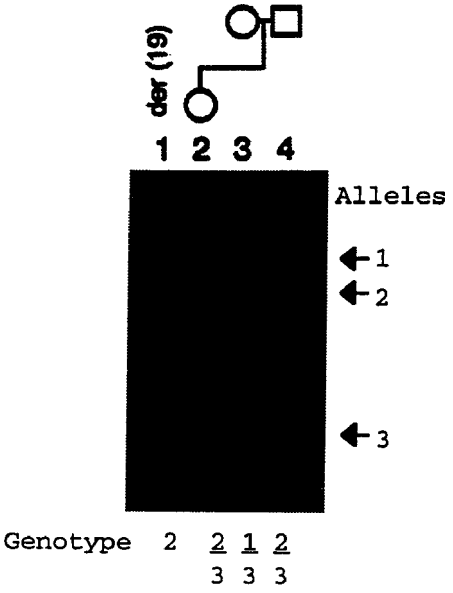
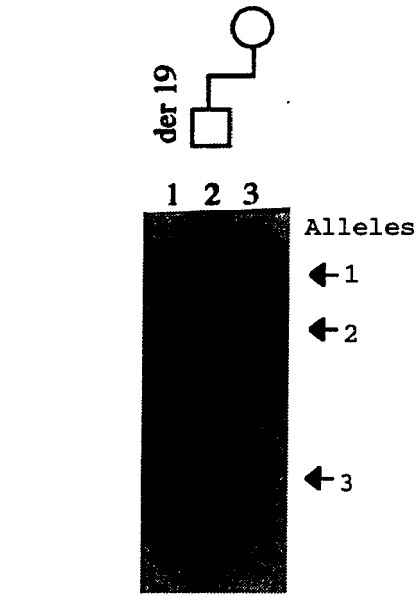
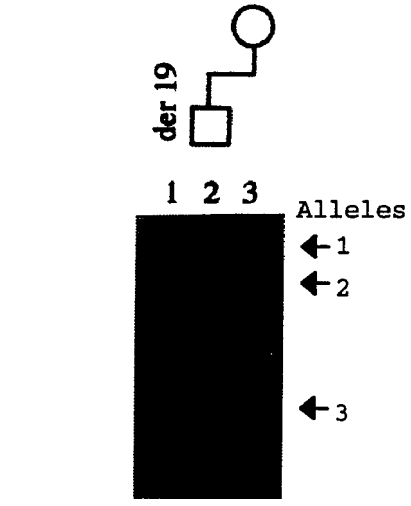

der (19)
1 2 3 4
Alleles
1
2
3
Genotype 2 2 1 2
3 3 3

Figure 23. Paternal origin of the chromosome 1 involved in the t(1;19) for patient 25AC. The autoradiograph from D1S158 showed allele 3 detected on the der(19) corresponded to the paternally derived allele. Because the paternal genomic DNA was unavailable, the patient's allele 3 was assumed to be paternally derived since allele 1 was maternally derived. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 25AC. Lane 3, Maternal genomic DNA.

Figure 24. Maternal origin of the chromosome 19 involved in the t(1;19) for patient 25AC. The autoradiograph from D19S49 showed allele 2 detected on the der(19) corresponded to the maternally derived allele. Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 25AC. Lane 3, Maternal genomic DNA.

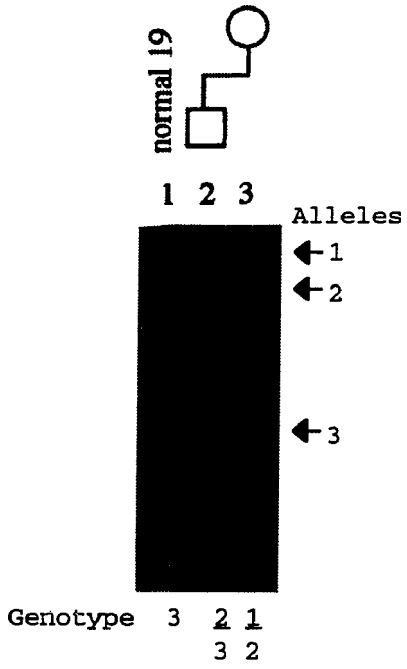


Genotype 3 $\frac{1}{3}$ $\frac{1}{2}$



Genotype 2 $\frac{2}{4}$ $\frac{1}{2}$

Figure 25. Paternal origin of the normal chromosome 19 homologue for patient 25AC. The autoradiograph from D19S49 showed allele 3 detected on the normal 19 corresponded to the paternally derived allele. Because the paternal genomic DNA was unavailable, the patient's allele 3 was assumed to be paternally derived since allele 2 was maternally derived. This result confirmed the maternal origin of the chromosome 19 involved in the t(1;19). Lane 1, Microdissected chromosome der(19). Lane 2, Genomic DNA from patient 25AC. Lane 3, Maternal genomic DNA.


normal 19
1 2 3
Alleles
1
2
3
Genotype 3 2 1
3 2

There were four possible parental origin combinations for t(1;19) (Figure 26). The translocated chromosome could have been formed by paternal 1 (P1) and paternal 19 (P19),

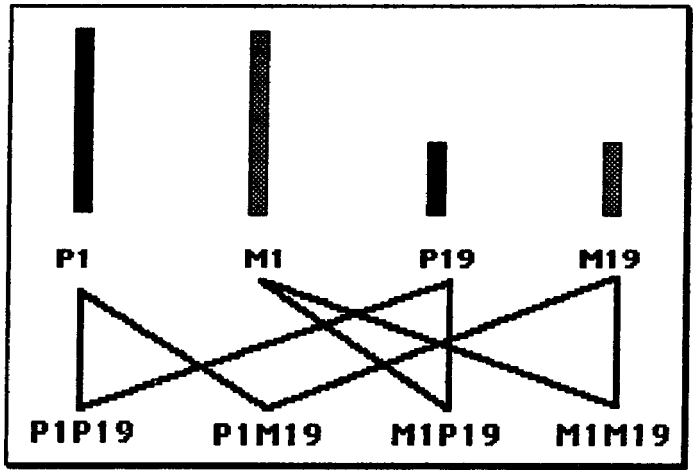


Figure 26. Four possible parental origin combinations for the t(1;19) chromosome. P: paternal. M: maternal.

maternal 1(M1) and maternal 19 (M19), P1 and M19, or M1 and P19. On the basis of the observation that a genomic imprinting effect is a qualitative event rather than a quantitative one the null hypothesis was: all four combinations had an equal probability to be represented in the translocation (H_0: $P_1=P_2=P_3=P_4=1/4$). The alternative hypothesis was: one of the combinations has a probability of close to 1 (H_a: $P_i \approx 1$, $i = 1,2,3,4$). Since genomic imprinting has been considered to be a qualitative phenomenon, observation of even one conflicting result would favor the null hypothesis and exclude a genomic imprinting influence on the translocation. However, to reject the null hypothesis, all cases studied should have the same parental origin for one or both chromosomes.

The final results for the eight patients studied are shown in Table 8. One particular combination of the two chromosomes was not found. Instead, all the possible combinations occurred at least once. Parental origin of the chromosome 1 involved in the t(1;19) translocation appeared random. Four patients had translocations with maternally derived chromosomes 1, and 4 with paternally derived chromosomes 1. Therefore, a parental origin bias was not observed for the chromosome 1. However, 6 of the 8 chromosomes 19 were paternally derived and only 2 maternally derived (Table 9). Using the binomial expansion to predict the probability of coincidence of random events where p (paternal origin) = q (maternal origin) = 1/2, the probability of the 6:2 combination occurring at random in this sample was p = 28/256 (~0.1 or 10%). In other words, there was a reasonable chance that this trend of paternal origin for chromosome 19 has occurred at random, so conclusive evidence for a paternal origin bias was not established. Furthermore, 2 chromosomes 19 were maternally derived, so two conflicting results have been observed. In this case, the original null hypothesis was not rejected, and the t(1;19) in childhood ALL was not influenced by genomic imprinting.

TABLE 8

Parental Origin of Chromosomes Involved in the t(1;19).

Patient	Chromosome 1	Chromosome 19
01JV.....	maternal	paternal
04RC.....	maternal	paternal
07PH.....	paternal	paternal
10JM.....	maternal	maternal
13YM.....	paternal	paternal
16HS.....	paternal	paternal
19ME.....	maternal	paternal
25AC.....	paternal	maternal

Table 9

Parent of Origin Bias of Chromosomes 1 and 19 Involved in the t(1;19).

	Chromosome 1	Chromosome 19
Maternal Origin	4	2
Paternal Origin	4	6

DISCUSSION

Chromosome Microdissection PCR (CMPCR) as a Method of Choice for Parental Origin Studies

The prerequisite for parental origin studies is to be able to analyze polymorphic markers that are only associated with the translocated homologues. This requires a physical separation of a normal homologue from its derivative homologue. This separation, however, is impossible for traditional molecular methods such as Southern-blot or quantitative PCR, because once DNA is extracted from a bone marrow sample, it would not be possible to recognize which allele is associated with the normal or the derivative homologue. The markers must also be polymorphic so that maternal and paternal alleles can be distinguished. Cytogenetic polymorphisms as markers are not, in general, very informative and this type of analysis can be subjective.

Chromosome microdissection PCR, as a molecular-cytogenetic method, provides the most direct and simple approach for parental origin studies of somatic translocations. The advantage of chromosome microdissection is that it allows for the isolation of aberrant chromosomes from the normal homologues for separate molecular analysis. Alternative molecular methods include isolation of translocation products into somatic cell hybrids before molecular analysis and long range PCR across the

translocation breakpoints. The production of somatic cell lines containing only the translocated homologue of interest can be time consuming and costly for studying multiple patients. Recently, long range PCR has enabled amplification across stretches of DNA greater than 30 kb in length (Barnes 1994) and has been used to study the parental origin of the chromosome 9 involved in the t(9;22) in CML (Melo et al. 1994). Long range amplification across the breakpoints of translocations can only be useful if: 1) the breakpoints cluster within the limits of long range PCR, 2) genomic sequences flanking the breakpoints have been determined, and 3) an informative polymorphism is located within the PCR product. Only the CMPCR approach allows for analysis of a highly polymorphic marker from separate homologues.

In addition, the CMPCR technique provided a more objective method for parental origin studies of the t(1;19). The analysis using highly polymorphic dinucleotide repeat loci avoided problems such as subjectivity associated with cytogenetic polymorphisms and relatively low informativeness of restriction site polymorphisms. Because of the high polymorphic information content (PIC) of dinucleotide repeat loci, patient selection for the t(1;19) study was independent of the presence or absence of a specific polymorphism and results were obtained on all patients with sufficient cytogenetic material using only a few loci (2 from chromosome 1 and 3 from chromosome 19). The CMPCR method using dinucleotide repeat loci was the best approach for parental

origin analysis of a rare translocation, such as the t(1;19), with limited number of patients. At the same time, the technique allowed for analysis of an unbiased sample since the first available t(1;19) patients were all included.

Technical Difficulties of CMPCR

The quality and quantity of the cytogenetic material from ALL patients presents some difficulties in using the CMPCR technique for the parental origin studies of the t(1;19). Generally, the ALL bone marrow samples have a low mitotic index and poor chromosome morphology. Storage of the cell pellet for extended periods of time further reduces the ability to obtain well spread metaphases and adequate banding of the chromosomes. This poor morphology and low mitotic index makes it necessary to scan numerous slides to obtain a few chromosomes for microdissection.

In addition, the quality of microdissected chromosomal DNA is effected by exposure to acid fixation during the cytogenetic preparation. The chromosomal DNA exposed to acid fixative will become depurinated at a frequency as high as 1 in 100 base pairs and the depurination is time dependent. Therefore the longer the cell pellet is stored, the higher the frequencies of depurination sites (Wahl et al. 1979; Brown and Greenfield 1987). These apurinic sites can cause an infidelity of the polymerase and termination of synthesis (Schaaper et al. 1983). Minimizing exposure of chromosomal DNA to acid fixative should decrease the likelihood of depurination. Slides used for microdissection should be

prepared from the cell pellet within a few hours of initial exposure to acid fixative. Unfortunately, the t(1;19) patient material consisted mainly of archived cell pellet, so minimizing exposure to acid fixative was not an option.

Difficulties achieving consistent amplification with the CMPCR method was most likely due to a combination of the factors described above. Low copy number of the original template and degradation of the chromosomal DNA decreased the chance that the specific stretch of target DNA would be intact and successfully amplified during the initial attempt.

Improvement of the CMPCR Technique

Great effort had been made to develop a more sensitive and consistent method for amplification of microdissected chromosomal DNA. Several techniques have been tried, including the use of nested and hemi-nested primers sets and random preamplification of the target DNA before loci specific amplification.

The use of nested primer sets for the dinucleotide repeat loci allowed for two rounds of PCR amplification which increased the sensitivity as well as specificity. The annealing temperature of the primer set in the first round was lowered to assure annealing to the limited number of templates. Then, the annealing temperature of the primer set in the second round was increased to obtain amplification only from the specific locus. Therefore, background amplification from the first set of primers would not be amplified when a new set of primers was used.

However, non-specific product in the second round of amplification was frequently a problem even with nested and hemi-nested primer sets.

Pre-amplification of the der(19) did account for successful loci specific amplification in almost half of the patient samples. This allowed for multiple loci specific amplifications from a single microdissection experiment. None of the three pre-amplification techniques tried were successful consistently. Perhaps, pre-amplification using primers with 3' sequences found at a high frequency in the human genome might provide a more powerful technique for randomly amplifying microdissected chromosomal DNA (Han et al. 1994a, 1994b). These primers have been used to randomly amplify genomic DNA. Subsequent fluorescence in situ hybridization of the product to metaphase chromosomes found them to produce better coverage of the DNA than primers using 3' random sequences (Han personal communication).

Parental Origin of the t(1;19) Chromosome with Childhood ALL

Eight children with pre-B cell ALL were evaluated for parental origin of the chromosomes involved in the t(1;19)(q23;p13) to determine if a genomic imprinting effect existed. Parental origin for chromosome 1 was random, with 4 maternal origin and 4 paternal origin. Therefore, a parental origin bias does not exist for chromosome 1. For chromosome 19, 6 of the 8 patients were paternal in origin. These results represented a preferential (but not exclusive)

participation of the paternal chromosome 19 in the translocation. Therefore, conclusive evidence for a parental origin bias for chromosome 19 was not established.

Parental origin studies only suggested the possibility of genomic imprinting, because an imprinting effect might best explain the bias. By definition, genomic imprinting is a cause and effect process, whereby the original imprint occurs in the gamete with secondary epigenetic modification occurring post-zygotically causing a functional difference in the embryo and adult. Parent-specific monoallelic expression, since it is one of the crucial hallmarks of imprinted genes, has been the functional difference studied most frequently. The classical concept of genomic imprinting implied that the two imprinted alleles were mutually exclusive in gene expression, where one would be actively transcribed while the other transcriptionally silenced. In a similar way, genomic imprinting may have influenced the formation of a somatic translocation with a specific parental origin by causing certain regions on one homologous chromosome to be more susceptible to a rearrangement. Actively transcribed genes are located in less condensed or 'open' regions of chromatin and these open regions of DNA are unusually accessible to damage (Alberts ed. 1989). If the accessibility of the DNA were unequal because of differential activity, genomic imprinting could have influenced the parental origin of the translocated chromosome by making one of the homologues more accessible. Conversely, the genomic imprint, causing

inactivity and a more condensed chromatin arrangement, might have influenced the parental origin by stabilizing one homologue making it inaccessible to a translocation event. Since an exclusive parental origin bias was not found for the t(1;19) chromosome, an imprinting effect in this particular subtype of childhood ALL seems unlikely.

An alternative explanation would be that a genomic imprinting effect does exist for chromosome 19 in childhood ALL but the 2 patients, 10JM and 25AC with maternal chromosome 19 origin, were different from the rest of the study group. The disparity detected in patients 10JM and 25AC might be explained by genetic heterogeneity seen among childhood ALL patients with the t(1;19). At the cellular level, cases of cytoplasmic Igμ heavy chain negative (cIg-) pre-B cell ALL with the t(1;19) have different translocation breakpoints than those in cIg+ cases (Privitera et al. 1992). Patients 10JM and 25AC were not immunologically different from the rest of the study group. Molecular variants of the t(1;19) have also been identified within the subgroup of cIg+ patients (Privitera et al. 1994). Therefore, further molecular analysis of the junction site of the translocation in these two patients might reveal a different rearrangement than those usually found in this subgroup.

Genomic imprinting does not appear to be directly involved in either the unidirectional formation of the t(1;19) chromosome or the disease-specific selection of one particular combination of chromosomes. Perhaps some mechanism

like one involved with the preferential loss of the maternal alleles in sporadic recessive tumor syndromes might be involved in a preferential expansion of those clones involving the paternal chromosome 19. Both combinations might have occurred initially but only the one involving chromosome 19 would be preferentially maintained as a clonal defect. Similar to the paternal genome producing excessive cell proliferation in complete hydatidiform moles (Austin and Hall 1992), the paternal chromosome 19 may have a greater effect on the malignant processes in childhood ALL.

It is also possible that more than one parental origin combination is being selected in different patients with the same translocation. In this case, the different parental origin pattern might be responsible for the clinical heterogeneity. Since all possible chromosomal combinations were found (Mat1Pat19, Mat1Mat19, Pat1Mat19, Pat1Pat19), it is unlikely that one specific combination of both chromosomes 1 and 19 could be correlated to specific clinical difference with such a small sample size. The trend of paternal origin for chromosome 19 (6 out of 8) might necessitate future study of a larger sample size to help establish an association between paternal origin of the chromosome 19 and clinical outcome as the disease progresses.

At the molecular level, the t(1;19) rearrangement in childhood ALL produces a hybrid E2A-PBX1 gene product with known oncogenic potential (Hunger et al. 1991; Kamps et al. 1991) and a mechanism of transformation has been suggested

(Lu et al. 1994; LeBrun and Cleary 1994). The identification of this fusion protein with oncogenic potential provides information about the initial transformation event in this specific childhood ALL. However, pursuing the possibility of a genomic imprinting effect in childhood ALL with the t(1;19) as well as other commonly recurring chromosome rearrangements, a much broader molecular area might be correlated with the disease state. Recent studies have indicated that the genomic imprinting process includes a complex involvement of both specific genes as well as chromosomal domains (Kitsberg et al. 1993; Nicholls 1994a; Knoll et al. 1994; LaSalle and Lalande, 1995; Neumann et al. 1995). As in some cases of the Beckwith-Wiedemann syndrome, when the maternally derived IGF2 gene (transcriptionally repressed) was translocated to another chromosome, large amounts of IGF2 were produced because the imprinted gene was translocated away from the domain responsible for the imprint (Hochberg et al. 1994). The genes involved directly in the t(1;19) rearrangement might not be under imprinting control; the effect of aberrant imprinting resulting from juxtaposition of two differently imprinted domains exerting 'position effects' on the adjacent genes may help explain variables involved in disease progression and remission.

The issue of imprinting has evolved into a complex one as more data has become available about the nature of imprinted genes. Most of the genes in mouse and humans unequivocally accepted to be imprinted have been found to be

widely expressed and have showed monoallelic expression in the majority of tissues (DeChiara et al. 1991; Bartolomei et al. 1991; Leff et al. 1992; Giannoukakis et al. 1993; Rainier et al. 1993; Glenn et al. 1993; Surani 1994). Differential methylation of DNA has been correlated with imprinting for all genes thus far analyzed and most likely has caused the differential transcription of the alleles (Razin and Ceder 1994). However, the correlation has not appeared to be a simple CpG methylation/transcriptional repression model. In some imprinted genes, DNA methylation has been associated with the transcriptionally silenced allele, while in others with the active allele (Li et al. 1993; Surani 1993). Some imprinted genes have even shown a tissue and developmental specific expression of the single allele (Davies 1994; Vu and Hoffman 1994; Jinno et al. 1994).

Genomic imprinting is a complicated process involving both local and regional imprinting factors such as DNA methylation, chromatin compaction, and other DNA sequence characteristics (Razin and Ceder 1994; Surani 1994; Rainier and Feinberg 1994; Karpen 1994; Neumann et al. 1995). These factors have effected gene expression, DNA replication patterns and quite possibly the formation of recurrent somatic translocations in a specific parent of origin manner (Kitsberg et al. 1993; LaSalle and Lalande 1995; Haas 1995). It remains uncertain whether a correlation between imprinting

and unidirectional translocation events in hematological malignancies exists (Melo et al. 1995; Haas 1995; Fioretos et al. 1995; Litz 1995).

Since an imprinting effect in the pre-B cell childhood ALL with the t(1;19) seems unlikely, similar investigations of other common recurring chromosome rearrangements found in leukemias may lead to a better understanding of the relationship between genomic imprinting and parental origin of somatic translocations. The study of imprinted genes or chromosomal regions involved in hematological malignancies should further the understanding of the molecular pathology of specific patient subgroups and help provide new information in clinical applications to improve diagnosis, treatment, and prognosis of the disease.

SUMMARY

The concept of genomic imprinting has changed our view of classical inheritance patterns by suggesting that two alleles with different parental origin may be differentially expressed. Increasing evidence, including parental origin bias of chromosomes involved in leukemia specific translocations, has suggested the involvement of genomic imprinting in the tumorigenic process.

Genomic imprinting has been previously described in other human diseases, but not before in childhood leukemia. Access to patients from across the United States through the Pediatric Oncology Group provided an opportunity to study this phenomenon in a rare type of childhood leukemia. The existence of parental origin bias of specific translocations found in hematological malignancies is a strong indication of genomic imprinting, since the bias must result from an unequal activity of the homologous chromosomes. Because these translocations originate somatically during the life of the individual, genomic imprinting might be involved either in the formation of the translocation or in its disease-specific selection.

A direct and simple approach for studying the parental origin of the translocated 1 and 19 chromosomes in this subgroup of childhood ALL patients was developed using a

molecular cytogenetic method, chromosome microdissection-PCR (CMPCR). The advantage of chromosome microdissection is that it allows for the isolation of aberrant chromosomes from the normal homologues for separate molecular analysis of highly polymorphic markers. Nested and hemi-nested primer sets were used to PCR amplify highly polymorphic dinucleotide loci, D1S158, D1S103, D19S49, D19S75 and APOC2, located on the derivative 19. Both direct PCR amplification from microdissected chromosomes as well as PCR amplification from product generated using several pre-amplification techniques were successful. The allele detected on the microdissected chromosomes was compared with parental alleles to assess the parent of origin of the chromosomes involved in the translocation.

Eight patients with the t(1;19)(q23;p13) in association with pre-B cell childhood acute lymphoblastic leukemia (ALL) were successfully studied. The ratio of paternal:maternal origin was 4:4 for chromosome 1 and 6:2 for chromosome 19. Evidence for a parental origin bias could not be established with this limited data set. The fact that 6 of 8 ALL patients with the t(1;19) have paternal origin of chromosome 19 suggested that additional studies and clinical follow-up were justified to help establish an association between paternal origin of the chromosome 19 and clinical outcome as the disease progresses.

Although genomic imprinting may not be involved in the formation of the t(1;19) chromosome found in this subgroup of

childhood ALL, application of our well established CMPCR procedure to study parental origin bias in other commonly recurring chromosome rearrangements might reveal a genomic imprinting effect in different subgroups. Genomic imprinting is a new frontier in cancer research. As more data about the precise mechanism of genomic imprinting becomes available, the relationship between a parental origin bias of somatic translocations and genomic imprinting may become apparent. Correlation of this information with clinical progression and outcome may help to improve the diagnosis and treatment of patients with hematological malignancies.

LIST OF REFERENCES

Austin A, Hall J (1992) Nontraditional inheritance. Ped Clinics of N America 39: 335-348

Alberts B (ed) (1989) Molecular biology of the cell, 2nd ed. Garland Publishing, New York.

Barch MJ (ed) (1991) The ACT cytogenetics laboratory manual, 2nd ed. Raven Press, New York.

Barlow DP, Stoger R, Herrmann BG, Saito K, Schweifer, N (1991) The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the tme locus. Nature 349: 84-87

Barnes WM (1994) PCR amplification of up to 35 kb DNA with high fidelity and high yield from lambda bacteriophage templates. Proc Natl Acad Sci 91: 2216-2220

Bartolomei M, Webber A, Brunkow M, Tilghman S (1993). Epigenetic mechanism underlying the imprinting of the mouse h19 gene. Genes and Dev 7: 1663-1673

Bartolomei MS, Zemel S, Tilghman, SM (1991) Parental imprinting of the mouse h19 gene. Nature 351: 153-155

Barton S, Ferguson-Smith A, Fundele R (1991) Influence for paternally imprinted genes on development. Development 113: 679-687

Bird, AP (1986) CpG-rich islands and the function of DNA methylation. Nature 321: 209-213

Bohlander S, Espinosa R, Le Beau M, Rowley J, Diaz M (1992) A method for the rapid sequence-independent amplification of microdissected chromosomal material. Genomics 13: 1322-1324

Brown SDM, Greenfield AJ (1987) A model to describe the size distribution of mammalian genomic fragments recovered by microcloning. Gene 55:327-332

Caron H, van Sluis P, van Hoeve M, de Kraker J, Bras J, Slater R, Mannens M, Voute PA Westerveld, Versteeg, R

(1993). Allelic loss of chromosome 1p36 in neuroblastoma is of preferential maternal origin and correlates with N-myc amplification. Nature Genet 4: 191-195

Carroll AJ, Crist WM, Parmley RT, Roper M, Cooper M, Finley WH. (1984) Pre-B cell leukemia associated with chromosome translocation 1;19. Blood 63: 721-724

Cattanach, BM (1986) Parental origin effects in mice. J Embryo Exp Morphl 97 [suppl]: 137-150

Cattanach B, Kirk M (1989) Differential activity of maternally and paternally derived chromosome regions in mice. Nature 315: 496-498

Cedar H, Razin A (1990) DNA methylation and development. Biochem Biophys Acta 1049: 1-8

Cedar H, Razin A (1994) DNA methylation and genomic imprinting. Cell 77: 473-476

Crist WM, Carroll AJ, Shuster JJ, Behm FG, Whitehead M, Vietti TJ, Look AT, Hahoney D, Ragab A, Pullen DJ (1990) Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13). A Pediatric Oncology Group study. Blood 67:117-122

Daley GQ, Ben-Neriah Y (1991) Implicating the bcr/abl gene in the pathogenesis of Philadelphia chromosome-positive human leukemia. Adv Cancer Res 57: 151-184

Davies SM (1994) Developmental regulation of genomic imprinting of the IGF2R gene in human liver. Cancer Res 54: 2560-2562

DeChiara, TM Robertson, EJ Efstratiadis, A (1991) Parental imprinting of the mouse insulin-like growth factor II gene. Cell 64: 849-859

Driscoll D, Waters M, Willams C, Zori R, Glenn C, Avidano K, Nicholls R (1992) A DNA methylation imprint, determined by sex of the parent distinguishes the Angleman and Prader-Willi syndromes. Genomics 13: 917-924

Dryja TP, Mukai S, Petersen R, Rapaport JM, Walton D, Yandell DW (1989) Parental origin of mutations of the retinoblastoma gene. Nature 339: 556-558

Eipers P, Barnoski BL, Han J, Carroll AJ, Kidd V (1991) Localization of the expressed human p58 protein kinase chromosomal gene to chromosome 1p36 and a highly related sequence to chromosome 15. Genomics 11: 621-629

Engle, E (1980) A new genetic concept: uniparental disomy and its potential effect. Am J Med Genet 6: 137-143

Ezzell, C (1994) Genomic imprinting and cancer. J NIH Res 6: 53-58

Feinberg, AP (1993) Genomic imprinting and gene activation in cancer. Nature Genet 4: 110-113

Fioretos T, Heisterkamp N, Groffen J (1994) No evidence for genomic imprinting of the human BCR gene. Blood 83: 3441-3444

Fioretos T, Heisterkamp N, Groffen J (1995) Standpoint on imprinting of BCR and ABL. Leukemia 9: 743-744

George AL, Knops JF, Han J, Finley WH, Knittle T, Brown GB (1993) Assignment of a human voltage-dependent sodium channel a-subunit gene (SCN6A) to 2q21-23. Genomics 19: 395-397

George AL, Varkony TA, Drabkin H, Han J, Knops JF, Finley WH, Brown GB, Ward DC, Haas M (1995) Assignment of a human heart tetrodotoxin-resistant voltage-gated Na^+channel a-subunit gene (SCN5A) to 3p21. Cytogenetics and Cell Genetics 68: 67-70

Giannoukakis N, Deal C, Paquette J, Goodyer C, Polychroonakos C (1993) Parental genomic imprinting of the human IGF2 gene. Nature Genet 4: 98-102

Giddings SJ, King C, Harman KW, Flood JF, Bishhof N (1992) Allele specific inactivation of insulin 1 and 2, in the mouse yolk sac, indicates imprinting. Nature Genet 6: 310-313

Glenn C, Porter KA, Jong M, Nicholls R, Driscoll D (1993) Functional imprinting and epigenetic modification of the human SNRPN gene. Hum Mol Gen 2: 2001-2005

Guan X, Trent JM, Meltzer PS (1993) Generation of band-specific painting probes from a single chromosome. Hum Mol Gen 2:1117-1121

Guillemot F, Caspary T, Tilghman SM, Copeland NG, Gilbert DJ, Jenkins NA, Anderson DJ, Joyner AL, Rossant J, Nagy A (1995) Genomic imprinting of Mash2, a mouse gene required for trophoblast development. Nature Genet 9:235-241

Haas O. (1995) Are ABL and BCR imprinted? No definitive answers, but more questions. Leukemia 9: 740-743

Haas O, Argyriou-Tirita A, Lion T (1992) Parental origin of chromosomes involved in the translocation t(9;22). Nature 359: 414-416

Hadano S, Watanabe M, Yokoi H, Kogi M, Kondo I, Tsuchiya H, Kanazawa I, Wakasa K, Ikeda JE (1991) Laser microdissection and single unique primer PCR allow generation of regional chromosome DNA clones from a single chromosome. Genomics 11: 364-373

Haig, D (1994) Is human insulin imprinted? Nature Genet 7: 10

Hall, J (1990) Genomic imprinting: review and relevance to human diseases. Am J Hum Gen 46: 857-873

Han J, Lu CM, Brown GB, Rado T (1991) Direct amplification of a single dissected chromosomal segment by polymerase chain reaction: a human brain sodium channel gene is on chromosome 2q22-q23. Proc Natl Acad Sci 88: 335-339

Han J, Knops JF, Huckaby C, Rauno G, Finley WH, Carroll AJ (1994a) Identification of a 1q23.3-q42.1 duplication using chromosome microdissection generated painting probes. Am J Hum Gen 55: A106

Han J, Zhu Z, Hsu CC, Finley WH (1994b) Selection of antisense oligonucleotides based on genomic frequency of the target sequence. Antisense Research and Development 4: 53-65

Hashimoto K, Azuma C, Ohashi K, Kamura S, Nobunaga T, Kimura T, Tokugwa Y, Kanai T, Saji F (1995) Loss of imprinting in choriocarcinoma. Nature Genet 9: 109-110

Hearne C, Ghost S, Todd J (1992) Microsatellites for linkage analysis of genetic traits. Trends in Genetics 8: 288-294

Heutink P, van der Mey AGL, Sandkuijl LA, van Gils APG, Bardoel A, Breedveld GJ, van Vliet M, van Ommen GJB, Cornelisse CJ, Oostra BA, Weber JL, Devilee P (1992) A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. Human Mol Gen 1: 7-10

Hirota T, Tsukamoto K, Deng HX , Yoshiura KI, Ohto T, Tohma T, Kiabe T, Harada N, Jinno Y, Niilawa N (1992) Microdissection of human chromosomal region 8q23.3-q24.11 and 2q33-qter: construction of DNA libraries and isolation of their clones. Genomics 13: 349-354

Hochberg A, Gonik B, Goshen R, deGroot N (1994) A growing relationship between genomic imprinting and tumorigenesis. Cancer Genet Cytogenet 73: 82-83

Hubert R, Weber J, Schmitt K, Zhang L, Arnheim N (1992) A new source of polymorphic DNA markers for sperm typing: analysis of microsatellite repeats in single cells. Am J Hum Genet 51: 985-991

Hudson TJ, Engelstein M, Lee MK, Ho EC, Rubenfield MJ, Adams CP, Housman DE, Dracopoli NC (1992) Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms. Genomics 13: 622-629

Hunger SP, Galili N, Carroll AJ, Crist WM, Link MP, Cleary ML (1991) The t(1;19)(q23;p13) results in consistent fusion of E2A and PBX1 coding sequences in acute lymphoblastic leukemias. Blood 77: 687-693

Jinno Y, Yun K, Nishiwaki K, Kubota T, Ogawa O, Reeve A, Niikawa N (1994) Nosaic and polymorphic imprinting of the WT1 gene in humans. Nature Genet 6: 305-309

Kalscheur VM, Marlman EC, Schepens MT, Rehder H, Ropers HH (1993) The insulin-like growth factor type 2 receptor gene is imprinted in mouse but not in human. Nature Genet 5: 74-78

Kao F (1990) Chromosome microdissection and microcloning in human molecular genetics. Som Cell Mol Genet 13: 375-380

Kao F, Yu J (1991) Chromosome microdissection and cloning in human genome and genetic disease analysis. Proc Natl Acad Sci 88: 1844-1848

Kamps MP, Look AT, Baltimore D (1990) The human t(1;19) translocation in pre-B ALL produces multiple nuclear E2A-PBX1 fusion proteins with differing transforming potentials. Genes Dev 5: 358-368

Karpen GH (1994) Position-effect variegation and the new biology of heterochromatin. Curr Opin Genet Dev 4:281-291

Katz F, Weber D, Gibbons B, Reeves B, McMahon C, Chessels J, Mitchell C (1992) Possible evidence for genomic imprinting in childhood acute myeloblastic leukemia associated with monosomy for chromosome 7. British J Haemat 80: 332-336

Kay GF, Barton SC, Surani MA, Rastan S (1994) Imprinting and X chromosome counting mechanisms determine Xist expression in early mouse development. Cell 77: 639-650

Kitsberg D, Sellg S, Brandels M, Simon I, Keshet I, Driscoll DJ, Nicholls RD, Ceder H (1993) Allele-specific replication timing of imprinted gene regions. Nature 364: 459-463

Knoll JHM, Nicholls RD, Magenis RE, Graham JM, Lalande M, Latt SA (1989) Angleman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 32: 285-290

Knoll JH, Cheng S, Lalande M (1994) Allele specificity of DNA replication timing in the Angleman/Prader-Willi syndrome imprinted chromosomal region. Nature Genet 6: 41-45

Knops JF, Han J, Carroll AJ, Finley WH (1993) A simplified method for amplification of polymorphic dinucleotide repeats from microdissected chromosomes. Am J Hum Gen 53: A1022

Knudson A (1971) Statistical study of retinoblastoma. Proc Natl Acad Sci 68: 820-823

Kruse PH, Patterson MK (eds) (1973) Tissue culture: methods and applications. Academic Press, New York

Lasalle JM, Lalande M (1995) Domain organization of allele-specific replication within the GABRB3 gene cluster requires a biparental 15q11-13 contribution. Nature Genet 9: 386-394

Leach R, Magewu A, Buckley J, Benedict W, Rother C, Murphee A, Griegel S, Rajeqsky M, Jones P (1990) Preferential retention of paternal alleles in human retinoblastoma: evidence for genomic imprinting. Cell Growth Diff 1: 401-406.

Leff S, Brannan C, Reed M (1992) Maternal imprinting of the Snrpn gene and conserved linkage homology with the human Prader Willi Syndrome region. Nature Genet 2: 259-264.

LeBrun DP, Cleary ML (1994) Fusion with E2A alters the transcriptional properties of the homeodomain protein PBX1 in t(1;19) leukemias. Oncogene 9: 1641-1647

Li E, Beard C, Jaenisch R (1993) Role for DNA methylation in genomic imprinting. Nature 366: 362-365

Litz CE, Copenhaver CM (1994) Paternal origin of the rearranged major breakpoint cluster region in chronic myeloid leukemia. Blood 83: 3445-3448

Litz CE (1995) The parental origin of the Philadelphia chromosome: evidence of additional, recurring recombinational events. Leukemia 9: 744-745

Lu CM, Han J, Rado T, Brown GB (1992) Differential expression of sodium channel subtypes in human brain. FEBS Lett 303: 53-58.

Lu Q, Wright DD, Kamps MP (1994) Fusion with E2A converts the PBX1 homeodomain protein into a constitutive transcriptional activator in human leukemias carrying the t(1;19) translocation. Mol Cell Bio 14: 3938-3948

Lüdecke HJ, Senger G, Classen U, Horsthemke B (1989) Banded chromosomes and enzymatic amplification. Nature 338: 349-350

Marx JL (1988) A parent's sex may affect gene expression. Science 239: 352-353

Mellentin JD, Nourse J, Hunger SP, Smith SD, Cleary ML (1989) Molecular analysis of the t(1;19) breakpoint cluster region in pre-B cell acute lymphoblastic leukemias. Genes Chromosomes Cancer 2: 139-247

Melo JV, Yan XH, Diamond J, Goldman JM (1994) Lack of imprinting of the ABL gene. Nature Genet 8: 318-319

Melo JV, Yan XH, Diamond J, Goldman JM (1995) Balanced parental contribution to the origin of the disrupted ABL gene and to ABL expression in chronic myeloid leukemia. Leukemia 9: 734-745

Meltzer P, Guan XY, Burgess A, Trent JM (1992) Rapid generation of region specific probes by chromosome microdissection and their application. Nature Genet 1: 24-28

Monk M (1987) Memories of mother and father. Nature 328: 203-204

Monk M (1988) Genomic imprinting. Genes Devel 2: 921-925

Neumann B, Kubicka P, Barlow DP (1995) Characteristics of imprinted genes. Nature Genet 9: 12-13

Nicholls RD, Knoll J, Butler MJ, Karam S, Lalande S (1989) Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. Nature 342: 281-285

Nicholls RD (1994a) New insights reveal complex mechanisms involved in genomic imprinting. Am Jour Hum Gen 54:733-740

Nicholls RD (1994b) Imprinting: the embryo and adult point of view. Trends in Genet 10: 389

Nourse J, Mellentin JD, Galili N, Wilkinson J, Stanbridge E, Smith SD, Cleary ML (1990) Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. Cell 60: 535-545

Ogawa O, Eccles MR, Szeto J, McNoe LA, Yun K, Maw MA, Smith PJ, Reeve AE (1993) Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms tumor. Nature 362: 749-751

Overbeck L, Weston M, Kimberling W, Johnson D (1992) A highly polymorphic dinucleotide $(CT)_n$ repeat polymorphism D1S158 on chromosome 1q isolated by microdissection. Human Mol Gen 1: 141

Pal N, Wakey R, Buckle B, Yeomans E, Pritchard J, Cowell J (1990) Preferential loss of maternal alleles in sporadic Wilms tumor. Oncogene 5: 1665-1668

Pettenati MJ, Haines JL, Higgins RR, Wappner RS, Palmer CG, Weaver DD (1986) Wiedemann-Beckwith syndrome: Presentation of clinical and cytogenetic data on 22 new cases and review of the literature. Hum Genet 74: 143-154

Privitera E, Kamps MP, Haysahi Y, Inaba T, Shapiro LH, Raimondi SC, Behm F, Hendershot L, Carroll AJ, Baltimore D, Look AT (1992) Different molecular consequences of the 1;19 chromosomal translocation in childhood B-cell precursor acute lymphoblastic leukemia. Blood 79: 1781-1788

Privitera E, Luciano A, Ronchetti D, Arico M, Santostasi T, Basso G, Biondi A (1994) Molecular variants of the 1;19 chromosomal translocation in pediatric acute lymphoblastic leukemia (ALL). Leukemia 8: 554-559

Pui C, Crist W, Look T (1990) Biology and clinical significance of cytogenetic abnormalities in childhood ALL. Blood 76: 1449-1463

Rainier S, Johnson LA, Dobry CJ, Ping AJ, Grundy PE, Feinberg AP (1993) Relaxation of imprinted genes in human cancer. Nature 362: 747-749

Rainier S, Feinberg A (1994) Genomic imprinting, DNA methylation and cancer. J Natl Cancer Inst 86: 753-759

Raimondi SC (1993) Current status of cytogenetic research in childhood acute lymphoblastic leukemia. Blood 81: 2237-2251

Razin A, Ceder H (1994) DNA methylation and genomic imprinting. Cell 77: 473-476

Reik W, Collick A, Norris ML, Barton SC, Surani MA (1987) Genomic imprinting determines methylation of parental alleles in transgenic mice. Nature 328: 248-251

Reik, W (1989) Genomic imprinting and genetic disorders in man. Trends in Genetics 5: 331-336

Reik W, Surani MA (1989) Genomic imprinting and embryonal tumors. Nature 338: 112-113

Riggins GJ, Zhang F, Warren ST (1994) Lack of imprinting of BCR. Nature Genet 6:226

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Schaaper RM, Kunkel TA, Loeb LA (1983) Infidelity of DNA synthesis associated with bypass of apurinic sites. Proc. Natl Acad Sci USA 80:487-491

Schroeder W, Chao L, Dao D, Strong L, Pathak S, Riccardi V, Lewis W, Sanders G (1987) Nonrandom loss of maternal chromosome 11 alleles in Wilms tumor. Am J Hum Genet 40: 413-420

Scrable H, Cavanee W, Ghavimi F, Lovell M, Morgan K, Sapienza C (1989) A model for embryonal rhabdomyosarcoma tumorigenesis that involves genomic imprinting. Proc Natl Acad Sci 86: 7480-7484

Searle AG, Beechey CV (1978) Complementation studies with mouse translocations. Cytogenet Cell Genet 30: 282-303

Searle AG, Peters J, Lyon M, Hall J, Evan E, Edwards J, Buckle V (1989) Chromosome maps of man and mouse. IV. Ann Hum Gen 53: 89-140

Senger G, Lüdecke HJ, Horsthemke B, Clausen U (1990) Microdissection of banded human chromosomes. Hum Genet 84: 507-511

Smeets D, Hamel BCJ, Helen MR (1992) Prader-Willi syndrome and Angelmann syndrome in cousins from a family with a translocation between chromosomes 6 and 15. N Engl J Med 326: 807-811

Soltzer D (1988) Differential imprinting and expression of maternal and paternal genomes. Ann Rev Genet 22: 127-146

Spence J, Perciaccante R, Greig G, Willard H, Ledbetter D, Hejtmancik J, Pollack M, O'Brian W, Beaudet A (1988) Uniparental disomy as mechanism for human genetic disease. Am J Hum Genet 42: 217-226

Spielvogel H, Hennies H, Clausen U, Washington S, Chakravarti A, Reis A (1992) Band specific localization of the microsatellite a D13S71 by microdissection and enzymatic amplification. Am J Hum Genet 40: 1031-1037

Stöger R, Kubicka P, Lui CG, Kafri T, Razin A, Ceder H, Barlow DP (1993) Maternal-specific methylation of the imprinted mouse Igf2r locus identifies the expressed locus as carrying the imprinting signal. Cell 73: 51-71

Surani MA (1986) Evidences and consequences of differences between maternal and paternal genomes during embryogenesis in the mouse, In: Rossan J, Pederson RA (eds) Experimaental approaches to mammalian embryonic development. Cambridge University Press, Cambridge, pp 401-435

Surani M, Reik W, Allen N (1988) Transgenes as molecular probes for genomic imprinting. Trends in Genetics 4: 59-62

Surani MA (1993) Silence of the genes. Nature 366:302-303

Surani MA (1994) Genomic imprinting: control of gene expression by epigenetic inheritance. Curr Opin Cell Biol 6: 390-395

Swain JL, Stewart TA, Leder P (1987) Parental legacy determines methylation and expression of an autosomal transgene: a molecular mechanism for parental imprinting. Cell 50:719-729

Temple IK, James R, Crolla J, Sitch F, Jacobs P, Howell WM, Bets P, Baum JD, Shields J (1995) An imprinted gene for diabetes? Nature Genet 9: 110-112

Thomas BJ, Rothstein R (1991) Sex, maps and imprinting. Cell 64:1-3

Toguchida J, Ishizaki K, Sasaki M, Nakamura Y, Ikenega M, Kato M, Sugimor M, Kotuora Y, Yamamuro T (1989) Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature 338: 156-158

van der Mey APC, Maawinklle-Mooy P, Cornelisse C, Schmidt C, van de Kamp J (1989) Genomic imprinting in hereditary paragangliomas; evidence for a new genetic theory. Lancet, ii: 1291-1294

van Gils AGA, van der Mey APC, Hoogma R, Sandkuijl L, Maaswinkle-Mooy P, Falke T, Pauwels E (1992) MRI screening of kindred at risk of developing paragangliomas: support for genomic imprinting in hereditary glomus tumours. Br J Cancer 65: 903-907

Viljoen D, Ramesar R (1992) Evidence for paternal imprinting in familial Beckwith-Wiedemann syndrome. J Med Genet 29: 221-225

Vu TH, Hoffman AR (1994) Promoter specific immprinting of the human insulin-like growth factor-II gene. Nature 371: 714-717.

Wahl GM, Stern M, Stark ER (1979) Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl paper and rapid hybridization by using dextran sulfate. Proc Natl Acad Sci 76: 3683-3687

Weber J, Kappel C, Kwitek A, May P (1990a) Dinucleotide repeat polymorphism at the D19S75 locus. Nucleic Acids Res 18: 4639

Weber J, Kappel C, May P (1990b) Dinucleotide repeat polymorphism at the D19S49 locus. Nucleic Acids Res 18: 1927

Weber J, Kwitek A, May P (1990c) Dinucleotide repeat polymorphism at the D1S103 locus. Nucleic Acids Res 18: 2199

Weber J, May P (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44: 388-396

Xu Y, Goodyer CG, Deal C, Polychronakos C (1993) Functional polymorphism in the parental imprinting of the human IGF2R gene. Biochem Biophys Res Comm 197: 747-754

Zhang L, Cui X, Schmitt K, Hubert R, Navidi W, Arnheim N (1992) Whole genome amplification from a single cell: implications for genetic analysis. Proc Natl Acad Sci 89: 5847-5851

APPENDIX

Table 10

Complete cytogenetic diagnosis of the patients with childhood ALL

Patient	Cytogenetic Diagnosis
01JV	46,XY[7]/ 46,XY,-19,+der(19)t(1;19)(q23;p13)[13]/ 46,XY, -19,+der(19)t(1;19)(q23;p13),dup(1)(q23q42),del(11)(q23)[1]
03RC	46,XX[3]/ 46,XX, der(19)t(1;19)(q23;p13)[17]
07PH	46,XY[10]/ 47,XY,t(1;19)(q23;p13),+i(1)(q10)[10]
10JM	46,XY[8]/ 47,XY,t(1;19)(q23;p13),del(13q),+21[12]
13YM	46,XX[1]/ 46,XX,-19,+der(19)t(1;19)(q23;p13)[19]
16HS	46,XY[5]/ 46,XY,-19,+der(19)t(1;19)(q23;p13)[16]
19ME	45,XX,t(13q14q)c[6]/ 46,XX,t(1;19)(q23;p13),t(13q14q)c[5]
22CC	46,XX[17]/46,XX,t(1;19)(q23;p13)[5]/46,X,-X,t(1;19)(q23;p13), +derdic(1)t(X;1)(q26;p11)[3]/47,XX,t(1;19)(q23;p13),+der(1)t(1;?) (p11;?)[4]
25AC	46,XY,-19,+der(19)t(1;19)(q23;p13)[],46,XY,i(7q), -19,+der(19)t(1;19)(q23;p13)[]

Table 11

Demographic data on t(1;19) childhood ALL patients included in the study.

Patient	Sex	Race*	Age at diagnosis	Geographic Loaction
01JV	M	H	11 years	San Antonio, TX
03RC	F	W	3 years	Morgontown, WV
07PH	M	-	1 month	Atlanta, GA
10JM	M	W	14 years	Stony Brook, NY
13YM	F	H	8 years	Sacramento, CA
16HS	M	W	1 year	San Diego, CA
19ME	F	W/H	4 years	Miami, FL
22CC	F	W	3 years	Winston-Salem, NC
25AC	M	B/W	5 years	Wichita, KS

* W: White; H: Hispanic; B: Black; -: unavailable

GRADUATE SCHOOL
UNIVERSITY OF ALABAMA AT BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Judith F. Knops

Major Subject Medical Genetics

Title of Dissertation A Molecular Cytogenetic Study of Imprinting in Childhood Acute Lymphoblastic Leukemia (ALL) with the (1;19) Translocation

Dissertation Committee:

Daniel C. Cooper, Chairman

Andrew Carroll

Sara C. Finley

Wayne N. Finley

George B. Brown

Director of Graduate Program Wayne N. Finley

Dean, UAB Graduate School

Date 8/28/96