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**Trisomies in Hematologic Malignancies**

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# TRISOMIES IN
# HEMATOLOGIC MALIGNANCIES

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# ORIGINAL ARTICLES

This thesis is based on the following original articles, referred to in the text by their Roman numerals:

I.   PAULSSON K, FIORETOS T, STRÖMBECK B, MAURITZSON N, TANKE HJ, and JOHANSSON B. Trisomy 8 as the sole chromosomal aberration in myelocytic malignancies: a multicolor and locus-specific fluorescence in situ hybridization study. *Cancer Genet Cytogenet* 2003;140:66-69.

II.  PAULSSON K, HEIDENBLAD M, STRÖMBECK B, STAAF J, JÖNSSON G, BORG Å, FIORETOS T, and JOHANSSON B. High-resolution genome-wide array-based comparative genome hybridization reveals cryptic chromosome changes in AML and MDS cases with trisomy 8 as the sole cytogenetic aberration. Manuscript.

III. PAULSSON K, PANAGOPOULOS I, KNUUTILA S, JEE KJ, GARWICZ S, FIORETOS T, MITELMAN F, and JOHANSSON B. Formation of tri-somies and their parental origin in hyperdiploid childhood acute lymphoblastic leukemia. *Blood* 2003;102:3010-3015.

IV.  PAULSSON K, MÖRSE H, FIORETOS T, BEHRENDTZ M, STRÖMBECK B, and JOHANSSON B. Evidence for a single-step mechanism in the origin of hyperdiploid childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 2005;44:113-122.

# ABBREVIATIONS

| | |
|---|---|
| ALL | acute lymphoblastic leukemia |
| AML | acute myeloid leukemia |
| array CGH | array-based comparative genome hybridization |
| BAC | bacterial artificial chromosome |
| CFU-GEMM | colony-forming unit of the erythrocyte-macrophage-megakaryocyte |
| CIN | chromosomal instability |
| CMD | chronic myeloproliferative disorder |
| CML | chronic myeloid leukemia |
| CNP | copy number polymorphism |
| CT21 | constitutional trisomy 21 |
| CT8M | constitutional trisomy 8 mosaicism |
| dmin | double minutes |
| EAD | equal allele dosage |
| FISH | fluorescence in situ hybridization |
| LOH | loss of heterozygosity |
| M-FISH | multicolor-FISH |
| MAC | morphology-antibody-chromosomes |
| MDS | myelodysplastic syndromes |
| miRNA | microRNA |
| MTX | methotrexate |
| PAC | P1 artificial chromosome |
| PCP | partial chromosome paint |
| QF-PCR | quantitative fluorescent polymerase chain reaction |
| RT-PCR | reverse transcriptase-polymerase chain reaction |
| SKY | spectral karyotyping |
| SNP | single nucleotide polymorphism |
| UAD | unequal allele dosage |
| UPD | uniparental disomy |
| WBC | white blood cell count |
| WCP | whole chromosome paint |

# PREFACE

Acquired clonal chromosome aberrations are found in a large proportion of malignant hematologic disorders. During the last decades, it has become increasingly clear that these genetic changes are closely associated with leukemogenesis, being involved in transformation as well as in neoplastic evolution. In hematologic malignancies, the balanced rearrangements have been successfully investigated, resulting in an increased understanding of the molecular mechanisms underlying the leukemogenic process, an improved stratification into prognostic and morphologic subgroups, and, recently, to novel treatment strategies. Gains of chromosomes – e.g., trisomies – are equally common. However, in contrast to the balanced rearrangements, little is known about the biologic significance and pathogenetic impact of these changes or how they arise. The aim of the present thesis was to find the answers to some of these questions.

This thesis is divided into four sections. The first is an introduction to chromosome abnormalities in malignant hematologic disorders, specifically focusing on trisomies, whereas the second is a brief summary of the present investigation, including the methods used and the most salient results. The third comprises reviews of the two chromosome aberrations/karyotypic abnormality patterns studied in articles I – IV: trisomy 8 in acute myeloid leukemia/myelodysplastic syndromes and hyperdiploidy in childhood acute lymphoblastic leukemia, including discussions of the results from the present investigation. The final section contains the original articles (I – IV) on which this thesis is based.

Lund, August 2005

# INTRODUCTION

## HEMATOLOGIC MALIGNANCIES

Hematologic malignancies are neoplastic disorders that affect the blood-forming bone marrow and the peripheral blood. They involve either the myeloid or the lymphoid lineages and comprise chronic proliferative or dysplasia-associated disorders, such as chronic myeloid leukemia (CML), chronic myeloproliferative disorders (CMD), and myelodysplastic syndromes (MDS), as well as the acute myeloid (AML) and the acute lymphoblastic leukemias (ALL), which are characterized by an accumulation of immature hematopoietic cells – i.e., blasts – in the bone marrow and peripheral blood.[1] The diagnosis and the prognostication of these disorders are based on clinical findings, such as white blood cell count (WBC) and age, the morphology and the immunophenotype of the neoplastic hematopoietic cells, and the presence of genetic aberrations.[1] The latter are seen in all types of hematologic malignancies, and are the subject of the present thesis.

## CHROMOSOME ABERRATIONS IN HEMATOLOGIC MALIGNANCIES

### *Neoplasia is a genetic disorder*

Acquired clonal chromosome aberrations are found in a wide range of neoplastic disorders (Fig. 1).[2] During the last century, it has become increasingly clear that these anomalies lead to deregulation of the genetic control systems of the cell, resulting in transformation, tumor development and tumor evolution.[3] Today, we know that cancer is genetic disease – i.e., the occurrence of neoplasia, as well as the clonal evolution of malignant disorders, are the direct consequences of somatic mutations in the genome. This understanding has had profound ramifications, both for the clinical management of such disorders and for the understanding of the biologic mechanisms behind tumorigenesis. Thus, the questions why these abnormalities occur, how they arise, what their pathogenetic effects are, and, last but not least, how these effects may be counteracted, have received much attention during recent years.
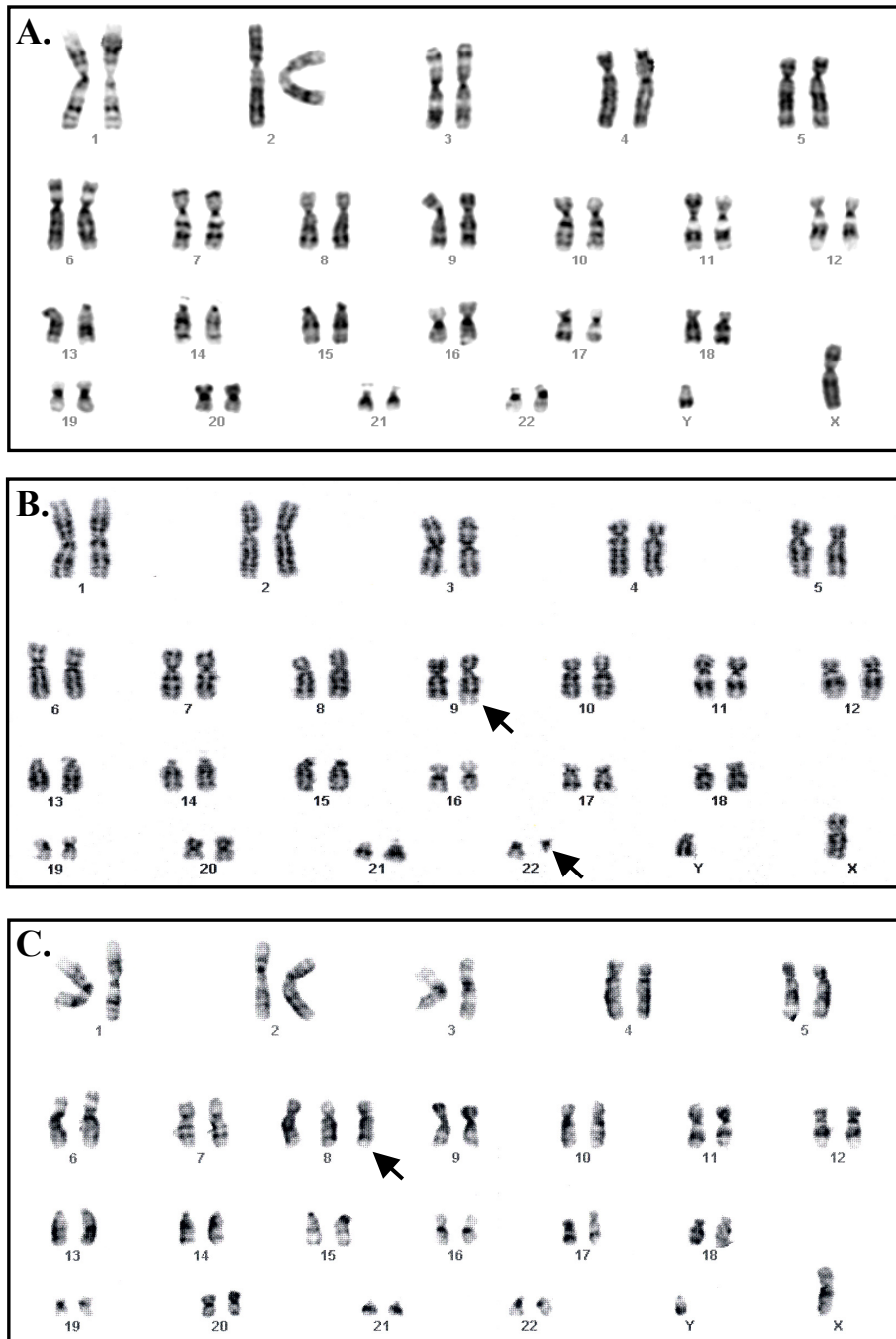
**Figure 1.** Examples of karyotypes. (A) Normal male karyotype, 46,XY. (B) Aberrant karyotype in a CML with the balanced rearrangement t(9;22)(q34;q11). (C) Aberrant karyotype in an AML with the unbalanced chromosome abnormality trisomy 8.

*Hematologic malignancies and chromosome abnormalities*

More than 30,000 cases of hematologic malignancies with chromosome aberrations have been reported to date, making it the most thoroughly cytogenetically investigated group of all neoplastic disorders.[4] This is partly due to the fact that the presence of different abnormalities has been shown to be of great clinical importance in hematologic malginancies.[5] For example, chromosome analysis is utilized for the classification of AML cases to different subtypes of this disorder – e.g., the t(15;17)(q22;q12) is found almost exclusively in acute promyelocytic leukemia (AML M3)[6] – and cases are assigned to prognostic risk groups and different treatment strategies based, in part, on the cytogenetic findings.[5] Furthermore, the understanding of the molecular mechanisms underlying leukemogenesis may lead to new treatments. Thus, a drug aimed directly at the abnormal fusion protein resulting from the CML-specific t(9;22)(q34;q11) was recently developed and has shown excellent results in clinical practice.[7] Taken together, cytogenetic and molecular genetic analyses are today invaluable for diagnostic, prognostic, and treatment-related purposes in malignant hematologic disorders.[5]

*Types of genetic aberrations*

The most characteristic karyotypic feature of hematologic malignancies – in particular of the acute leukemias and CML – is the presence of balanced translocations and inversions.[8] The pathogenetic impact of these abnormalities is today relatively well understood; two, or possibly three, biologic consequences have been described. First, balanced re-arrangements may lead to deregulation of a gene in one of the breakpoints by placing its coding sequence under the transcriptional control of regulatory elements in the other breakpoint.[9] For example, the t(8;14)(q24;q32), characteristic for Burkitt's lymphoma/leukemia, fuses the oncogene *MYC* with *IGH@*, causing overexpression of the former gene.[10] Secondly, they may result in fusion of the genes in the respective breakpoints, generating chimeric genes with novel functions.[9] In hematologic malignancies, genes encoding transcription factors and tyrosine kinases are commonly involved, such as the hematopoietic transcription factor gene *RUNX1*, which has several different fusion partners in AML, and the ABL1 tyrosine kinase, encoded by a gene which forms a chimera with *BCR* as a result of the t(9;22) in CML.[3,11] Thirdly, *RUNX1* has been reported to be translocated to out-of-frame transcripts, leading to its truncation.[12,13] Functional studies of one of these chimeras – the

*RUNX1/AMP19* formed by a t(19;21)(q13;q22) in secondary AML – showed that it blocked maturation of immature myeloid cells.[13] Thus, one possible pathogenetic impact of structural rearrangements is disruption of genes, presumably resulting in haploinsufficiency. However, further studies of this putative mechanism are needed. Although the above-mentioned chimeras frequently result from translocations and inversions, they may also be formed on extra-chromosomal episomes or through intra-chromosomal deletions.[14-17] Because these may be cytogenetically cryptic, it is as yet unknown how common the latter mechanisms are.

Imbalances, i.e., aberrations that result in gain or loss of genetic material, are even more common than translocations and inversions in hematologic malignancies. These include amplifications, duplications, hetero- or homozygous deletions, monosomies, and trisomies. Amplifications – multiple extra copies of a chromosome region – may occur in the form of heterogeneously staining regions or double minutes (dmin), and result in overexpression of one or more genes. Upregulation of genes is also a possible consequence of duplications, although these are low-copy number changes and are therefore expected to have a lesser impact on the gene expression patterns. Deletions may lead to loss of one or two copies of a tumor suppressor gene and also, as mentioned above, to fusion genes.[15-18] The pathogenetic impact of monosomies and trisomies are more elusive. Presumably, they result in dosage effects for a large number of genes. The specific problems regarding trisomies are further discussed below.

Gene mutations, including point mutations or partial segmental duplications, have also been described in many malignant hematologic disorders. These may result in upregulation of the gene, such as activation of *FLT3* in AML,[19] or in loss-of-function, e.g., haplo-insufficiency for *RUNX1* in AML.[20]

Recently, segmental uniparental disomies (UPDs) – i.e., parts of two homologues being derived from the same parental chromosome – have been described in AML. Two studies have identified such anomalies in 20% of investigated AML cases.[21,22] The selective advantage that these anomalies may confer to the cell is as yet unknown, but loss of a wild-type tumor suppressor gene with subsequent duplication of a pre-existing mutated allele is one possibility; de-regulation of imprinted loci another.[22]

Epigenetic alterations have also been reported to be common in hematologic malignancies, resulting in silencing of important regulatory genes, such as the cyclin-dependent kinase inhibitors *CDKN2A*

(previously *P16*, *INK4A*) and *CDKN2B* (previously *P15*, *INK4B*), thereby promoting leukemogenesis.[23]

Finally, recent findings implicate microRNAs (miRNA) in tumorigenesis. This family of non-coding RNAs negatively regulates the expression of genes, and their amplification or deletion could therefore result in deregulation of genes involved in the development of leukemia.[24] For example, underexpression of the miRNA genes *miR15* and *miR16* has been reported to result from deletions of the chromosome band 13q14 in chronic lymphocytic leukemia.[25]

## TRISOMIES IN HEMATOLOGIC MALIGNANCIES

Although balanced fusion gene-forming rearrangements may be the cytogenetic feature most commonly thought of in the context of hematologic malignancies, gains of chromosomes – e.g., trisomies – is an equally frequent finding. In AML, for example, +6, +8, +11, +13, +19, +21, and +22 are each found, as the single anomaly or in addition to other changes, in >2% of unselected cytogenetically abnormal cases – the by far most common being trisomy 8, which is present in approximately 15%.[2] Furthermore, in childhood ALL, the high hyperdiploid (>50 chromosomes) cytogenetic subgroup – characterized by a massive gain of specific chromosomes – constitutes almost half of all cytogenetically abnormal cases.[26] However, in spite of this frequent occurrence of trisomies – and in sharp contrast to the well-investigated biologic effects of fusion gene-forming rearrangements – next to nothing is known about their biologic outcome, their pathogenetic impact, or how they arise.

*Gene dosage effects of trisomies*
The occurrence of a trisomy results in duplication of hundreds of loci on the gained chromosome. It seems obvious that this must lead to dosage effects, not only for genes on the trisomic chromosome – which would be expected to display a 1.5 increase of expression – but also for loci on other chromosomes, which may be regulated by the protein products of the first group of genes. Thus, a specific chromosome-induced effect, as well as an overall deregulation of gene expression, should be seen.[27]

In fact, microarray gene expression analyses have shown that both of the above-mentioned effects occur as a consequence of trisomies in malignant hematologic disorders.[28,29] However, additional, cytogenetically cryptic, genetic aberrations may also be present in such cases,

and hence it cannot be excluded that hidden changes influence the observed gene expression signatures. Avoiding this problem, Upender et al.[30] used chromosome transfer to introduce trisomies 3, 7, and 13, respectively, into different cell lines, enabling investigation of the direct effect of gained chromosomes. Their results showed that introduction of an extra chromosome leads to a general increase in the expression of the genes residing on that chromosome, as well as to deregulation of a substantial proportion of the loci at other chromosomes. Investigations of cells from fetuses with constitutional trisomies 13 and 21 have also been performed.[31,32] These cells are not expected to harbor additional genetic anomalies, and can therefore be utilized to investigate how trisomies affect gene expression. Microarray expression analyses showed a general upregulation of chromosome 13 and chromosome 21 genes, respectively, and additional gene deregulation was detected in at least one study.[31,32]

Taken together, the presence of a trisomy results in large-scale gene dosage effects. However, although this gene deregulation is likely to be pathogenetically important, the question whether this is sufficient for leukemogenesis remains unanswered.

*Trisomies as sole cytogenetic aberrations – additional cryptic changes?*
Although trisomies are frequently seen as isolated chromosome aberrations in hematologic malignancies, this does not exclude the presence of additional, cytogenetically cryptic anomalies. For example, gene point mutations, uniparental disomies, and rearrangements involving telomeric sequences, such as the t(12;21)(p13;q22) in ALL,[33] are not detectable by standard G-banding analysis. In fact, it has been suggested that the gene dosage effects resulting from trisomies are not sufficient for leukemogenesis, but that specific primary rearrangements are also needed.[8] These could be present at the duplicated chromosome, as described below, or they could be at any other chromosome.

In this context, AML in children with Down syndrome is enlightening. These patients, who have trisomy 21 constitutionally (CT21), have a highly increased risk of developing myeloid malignancies, especially of the AML M7 subtype.[34] The pathogenetic mechanism behind this is unknown; because not all individuals with CT21 develop AML, +21 by itself cannot be sufficient for leukemogenesis. Recently, mutations in the *GATA1* gene at Xp11 have been identified in the vast majority of these AML cases.[35] However, since *GATA1* mutations also are present in most CT21 patients with transient myeloproliferative disorder – a preleukemic state which sometimes precedes the AML, but

which often resolves by itself without transformation – these cannot be sufficient for AML transformation.[34,36] Nevertheless, their detection confirms that hidden genetic aberrations may be present in cases with single trisomies.

Identification of additional genetic changes in hematologic malignancies with isolated trisomies would be important for several reasons. For example, cryptic anomalies may have a clinical impact. Also, the development of imatinib (Gleevec), which directly targets the BCR/ABL1 protein resulting from the t(9;22) in CML,[7] shows that drugs aimed specifically at the products from genetic rearrangements may become a reality in future treatment of malignancies. Last but not least, the detection of additional genetic abnormalities in cases with trisomies as the sole cytogenetic change would undoubtedly increase our under-standing of leukemogenesis.

*Trisomies – ways to duplicate specific loci?*
An intriguing possibility is that trisomies are associated with the dupli-cation of a mutated allele, a cryptic rearrangement, or an imprinted locus. If so, gain of a whole chromosome is, at least in part, simply a way to obtain more copies of a specific aberration/allele on that chromosome. The first experimental evidence for this mechanism came from induced mouse skin squamous cell carcinomas, in which a nonrandom duplication of the chromosome carrying a mutated *Hras1* allele was noted.[37] A few years later, Caligiuri et al.[38] reported that trisomy 11 in AML was associated with partial tandem duplications of *MLL* (previously *ALL1*). Surprisingly, subsequent studies showed that it was the chromosome 11 containing the wild-type *MLL* allele that was duplicated, excluding that the pathogenetic outcome of the trisomy was duplication of the mutated copy.[39] However, the initial finding spurred other researchers to search for aberrant oncogenes in trisomic chromosomes. Hence, duplications of the mutated genes *MET* in hereditary papillary renal carcinoma with +7, *KIT* in AML with +4, and *RET* in multiple endocrine neoplasia type 2-associated pheochromocytomas with +10 were identified.[40-43] In the latter disorder, some cases with disomy 10 displayed deletion of the wild-type allele, suggesting that the relative dosage of the mutated copy of *RET* was the pathogenetically important outcome.[43] In contrast, Kawakami et al.[44] found no changes in *MET* in testicular germ-cell tumors with +7 and Powell et al.[45] detected no internal tandem duplications of *FLT3* in AML with +13. Taken together, duplication of a mutated allele may be the functional outcome of some trisomies in malignant disorders. However,

they have so far only been identified in a subset of gained chromosomes, and many recurrent trisomies remain to be investigated.

It is also conceivable that a trisomy results in duplication of a cytogenetically undetectable fusion gene-forming rearrangement. A hypothetical example is the duplication of a cryptic der(8)t(8;8) with breakpoints in the same chromosome band, which may be misinterpreted as gain of a normal chromosome 8. The basis for this suggestion is that gain of an extra derivative chromosome containing a fusion gene is a common finding in hematologic malignancies, e.g., duplication of the Philadelphia chromosome – +der(22)t(9;22) –in CML.[2,46] However, gains of derivates containing the reciprocal – putatively without pathogenetic effects – chimeric genes are also frequent in some translocation-positive hematologic malignancies, suggesting that the resulting imbalance may be the biologically important outcome, and not the duplication of a fusion gene.[46] Nevertheless, a subset of the recurrent trisomies in hematologic malignancies may be derivatives containing cryptic rearrangements, although this mechanism has never been described.

Furthermore, the duplication of a chromosome may result in de-regulation of imprinted loci, i.e., of genes that are differentially expressed depending on whether they are maternally or paternally inherited.[47] This would be detectable as a preferential parental origin of the gained chromosome. Haas[48] suggested that this mechanism may be of importance in hyperdiploid childhood ALL; something that will be further discussed in the "Hyperdiploidy in childhood ALL" section below. Apart from investigations of the latter disease subgroup,[49,III,IV] no studies have specifically addressed the possibility of preferential parental duplications in hematologic malignancies with trisomies. However, the fact that some other genetic aberrations display skewed parental origin in neoplastic disorders – such as preferential loss of the maternally derived chromosome band 11p15 in Wilms' tumors and of the paternally inherited 19q in oligodendrogliomas[50,51] – as well as the recent reports of segmental UPDs in AML,[21,22] suggest that imprinting effects may be involved in tumorigenesis.

*The formation of trisomies*
Solid tumors are often characterized by massive aneuploidy, possibly resulting from a higher rate of obtaining chromosome aberrations; a phenomenon known as chromosomal instability (CIN).[52] In many instances, this has been associated with mitotic spindle aberrations and mutations in genes encoding proteins involved in the mitotic check-

points.[52] In contrast, hematologic malignancies are generally cyto-genetically stable, harboring only a few, nonrandom numerical abnormalities; they do not display CIN. It is, however, possible that the same pathways are involved in the formation of some trisomies in malignant hematologic disorders as in the occurrence of CIN in solid tumors. Thus, centrosome aberrations have been detected in AML and CML in blast crisis with cytogenetic abnormalities, including chromo-some rearrangements as well as numerical changes.[53,54] Furthermore, single cases of AML and ALL have been reported in patients with mosaic variegated aneuploidy, a condition caused by constitutional mutations in the mitotic spindle checkpoint gene *BUB1B*.[55-57] This gene has also been shown to be mutated in some T-cell leukemias with complex karyotypes.[58] Finally, occasional mutations in the mitotic check-point genes *CHEK2* (previously *CHK2*) in AML/MDS and *MAD1L1* in B-cell lymphomas have been described, but at low frequencies and without concordant numerical abnormalities, at least in the study by Hofmann et al.[59-61] The latter suggests that mutations in these genes are not associated with aneuploidy in hematologic malignancies. Taken together, the mechanisms behind the occurrence of trisomies in hemato-logic malignancies remain largely unknown.

# THE PRESENT INVESTIGATION

The articles constituting the basis of this thesis may be divided into two parts: studies I and II, in which we attempted to identify cryptic chromosome changes in AML and MDS with trisomy 8 as the sole cytogenetic aberration, and studies III and IV, in which the mechanisms behind the occurrence of hyperdiploidy and the possibility of imprinting effects in this karyotypic subgroup were investigated in childhood ALL. Below is a brief introduction to the methods used, as well as a short summary of the most salient findings. For details on how the individual studies were performed, see articles I – IV.

## MATERIALS AND METHODS

### Patients
All patients had been analyzed with standard cytogenetic techniques at the Department of Clinical Genetics, Lund University Hospital, Sweden as part of the clinical evaluation of their respective disorders. The present investigation comprised a total of 17 AML and MDS cases and one CMD with trisomy 8 as the apparently sole aberration. Furthermore, 37 childhood ALLs with hyperdiploidy were included. For 22 of these cases, samples from the patients' parents were obtained and analyzed. All studies were approved by the Research Ethics Committee of Lund University.

### Fluorescence in situ hybridization
Fluorescence in situ hybridization (FISH) is a technique in which fluorescently labeled probes are hybridized to target sequences in interphase or metaphase cells and visualized in a fluorescence microscope (Fig. 2).[62] The resolution of FISH depends on the utilized probes. For example, multicolor-FISH (M-FISH) – such as the combined ratio labeling probe set used in the present investigation – and spectral karyotyping (SKY) enable detection of each chromosome pair in a single hybridization,[63,64] generating information of the whole genome, whereas gene-specific and subtelomeric probes only provide data for the investigated loci – sometimes with a resolution as high as 10 kb – and the subtelomeric sequences of a chromosome arm, respectively (Fig. 2A, C). In between these extremes are FISH analyses performed with  whole  and  partial
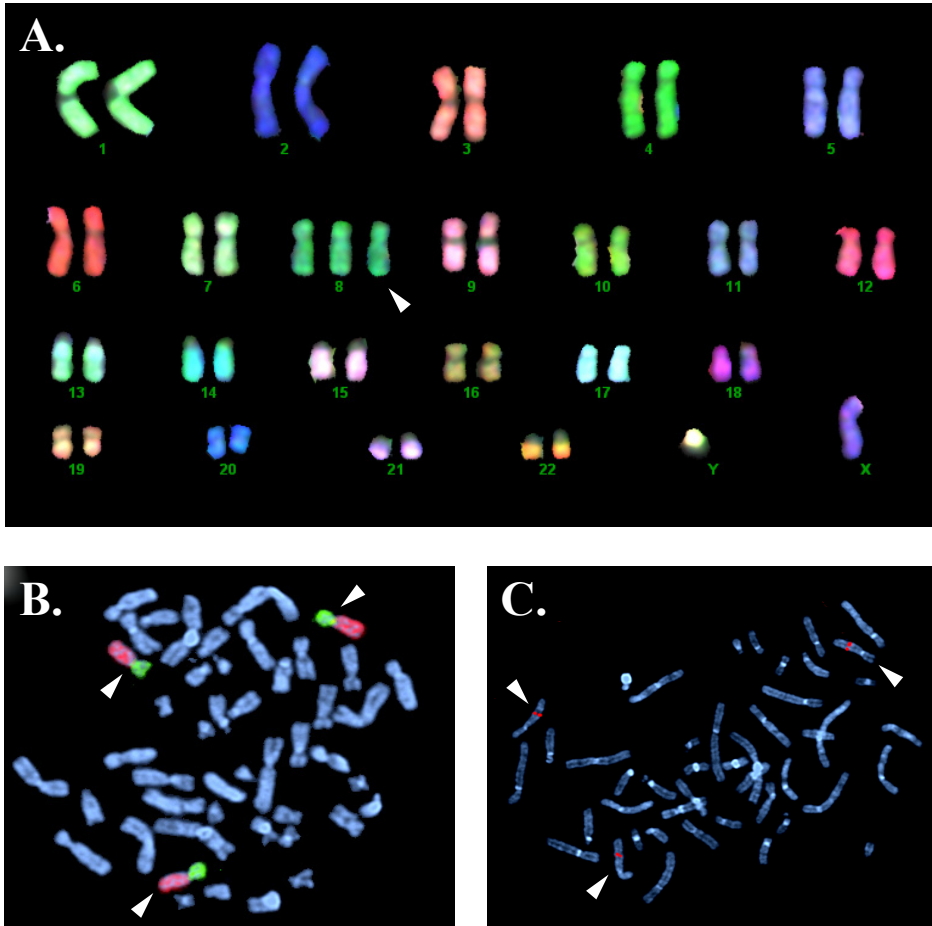
**Figure 2.** Examples of FISH findings in +8-positive AML/MDS in article I. Trisomy 8 is the only abnormality detected. Chromosomes 8 are indicated by arrows. (A) M-FISH. (B) FISH analysis with partial chromosome paint probes for 8p (green) and 8q (red). (C) FISH analysis with a gene-specific probe for *RUNX1T1* (red).

chromosome paint (WCP and PCP, respectively) probes, resulting in identifcation of chromosomes and chromosome parts (Fig. 2B), and multicolor banding/RxFISH, which generates chromosome-specific banding patterns, enabling detection of structural rearrangements within chromosomes.[68,69]

In hematologic malignancies, FISH-based techniques have been used for identification and characterization of chromosome aberrations. This method was utilized in investigations I, II, and IV. In study I, FISH analyses with probes for M-FISH, PCP and subtelomeric probes, and

locus-specific FISH for four genes were performed. In study II, bacterial and P1 artificial chromosomes (BACs and PACs, respectively) and gene-specific probes were utilized for FISH confirmation of intra-chromosomal gains and losses. In study IV, determination of chromosome copy numbers was done with M-FISH and interphase FISH.

*Array-based comparative genome hybridization*

In array-based comparative genome hybridization (array CGH) analyses, test and reference DNA is labeled with different fluorescent dyes and hybridized to slides containing genomic sequences, for example BAC clones.[65] This enables detection of gains and losses of genetic material, with a resolution level depending on the size of the clones and their spacing across the genome. In neoplastic disorders, array CGH may be used as a supplementary method to G-banding and FISH for detection and characterization of chromosome aberrations and for identification of cytogenetically cryptic abnormalities. In study II of the present investigation, array CGH was utilized for the latter of these purposes.

*Quantitative fluorescent PCR*

Quantitative fluorescent polymerase chain reaction (QF-PCR) with poly-morphic microsatellite markers is a rapid method for DNA identification and determination of chromosome copy numbers.[66] Primers for micro-satellite markers are fluorescently labeled and used in a semiquantitative PCR; the resulting products are separated by capillary electrophoresis. Alleles are visualized as peaks: heterozygotes display two allelic peaks, whereas homozygotes display one peak. Copy number changes of the chromosome region including the marker are detectable as increased or decreased peak-to-peak ratios. Originally, this method was used for paternity testing and forensic DNA analyses. During recent years, QF-PCR has emerged as an alternative to G-banding and FISH for prenatal detection of constitutional whole-chromosome imbalances, utilizing markers localized to the commonly aneuploid chromosomes.[66,67]

  Because QF-PCR may be used for both detection of chromo-some gains and (if parental samples are available) for determination of the parental origin of the chromosomes, this method was ideal for addressing the objectives of studies III and IV.

**RESULTS ARTICLES I/II**

The aim of studies I and II was to identify cryptic genetic aberrations in AML and MDS with trisomy 8 as the apparently sole chromosome abnormality. In the first of these investigations, FISH was used to search for rearrangements between chromosomes or within chromosome 8. No cytogenetically cryptic changes were found with M-FISH or metaphase FISH utilizing PCP and subtelomeric probes for 8p and 8q and probes specific for the chromosome 8-genes *FGFR1*, *MYST3* (previously *MOZ*), *RUNX1T1* (previously *ETO*), and *MYC* in thirteen cases (Fig. 2).

       Because small imbalances involving loci other than the above-mentioned genes would not be detectable by the FISH assays described in article I, the investigation was extended utilizing array CGH in study II. Analyses with this method revealed imbalances of 0.2 – 4.9 Mb, comprising segmental duplications and hemizygous deletions, in 9/10 AMLs/MDSs. In total, 23 different changes were identified, but eleven of these overlapped with previously described genomic copy number poly-morphisms (CNPs) and were hence most likely constitutional. The remaining twelve abnormalities were present in altogether five AMLs/MDSs. Although some of these imbalances may have been novel CNPs, at least two were undoubtedly leukemia-associated: a hemizygous deletion at 12p13, including the *ETV6* gene and similar to previously described losses in AML, and a hemizygous del(7)(p14p14), which was shown to have occurred prior to the trisomy 8. Thus, the findings presented in article II showed that cryptic imbalances are frequent in trisomy 8-positive AML and MDS, and that +8 may not be the primary event even when found as the sole cytogenetic aberration.

**RESULTS ARTICLES III/IV**

Studies III and IV had two aims: to investigate the mechanisms behind the formation of hyperdiploidy in childhood ALL and to address the possibility of imprinting effects related to the parental origin of the gained chromosomes or to the presence of UPDs.

       In study III, QF-PCR with polymorphic markers was utilized to investigate ten hyperdiploid childhood ALLs. We showed that the hyper-diploid pattern in these cases most likely had arisen by a simultaneous gain of chromosomes in a single abnormal cell division. However, a prerequisite for definite determination of the basis of multiple chromo-

some gains with this method is that all disomic and tetrasomic chromosomes are identified and investigated. Because the G-banding morphology was poor in the included cases – as it usually is in this cytogenetic subtype – and samples for FISH were not available, this requirement could not be fulfilled. An attempt was made to use conventional CGH for identification of gains, but the results were ambiguous. Therefore, we could not exclude that the hyperdiploidy had arisen via a tetraploid state or by sequential gains of chromosomes in consecutive mitoses based on the results presented in article III, although these alternatives were less likely. Hence, study IV was designed to resolve this issue: cases were selected on the basis of material being available for FISH and were investigated with M-FISH if analyzable metaphases were present and otherwise with interphase FISH for the commonly gained chromosomes. This approach resulted in identification of all disomies and tetrasomies in ten cases and of all tetrasomies in the remaining seventeen, enabling definite conclusions regarding the origin of the hyperdiploidy in the first ten cases and strong indications of the most likely mechanism in the remaining. The results provided strong evidence for the simultaneous gain of chromosomes suggested by study III.

Possible imprinting effects for the gained chromosomes were addressed by investigation of hyperdiploid childhood ALLs and samples obtained from the patients' parents in study III. These parental samples had been collected in the early 1990's but not investigated previously because of lack of a rapid technique for identification of the origin of chromosomes until QF-PCR with polymorphic markers became available. Although no statistically significant ratios of parental origin were detected, trends toward preferential duplication of the paternal chromosome 8 and the maternal chromosome 14 were seen. However, the limited number of cases precluded definitive conclusions. In study IV, samples were collected from the parents to an additional twelve patients with hyperdiploid childhood ALL and trisomies 8 or 14. The subsequent QF-PCR analyses revealed no preferential paternal or maternal origin, respectively, excluding imprinting effects related to the parental origin of gained chromosomes in hyperdiploid childhood ALL.

Finally, the possibility of a pathogenetic impact of UPDs for the frequently gained chromosomes in hyperdiploid childhood ALL was addressed in study IV. Only one UPD, not associated with the formation mechanism, was identified among the 27 cases. Thus, UPDs are not common for these chromosomes in hyperdiploid cases.

# REVIEWS

## TRISOMY 8 IN AML AND MDS

Trisomy 8 is one of the most common cytogenetic findings in myeloid malignancies, including AML, MDS, CMD, and CML.[2] In spite of this, the biologic importance and the functional – and pathogenetic – outcome of this aberration remain elusive. Below, our present knowledge of +8 is summarized, focusing on trisomy 8 as the sole anomaly in AML and MDS and including the results presented in articles I and II.

*Epidemiology*
A survey of published, unselected karyotypically aberrant AML and MDS cases shows that trisomy 8 as the single cytogenetic change is present in approximately 6% and 11%, respectively (Tables 1, 2). Similar incidences have previously been reported in studies of cytogenetic subgroups in AML and MDS.[70-75] This makes +8 the most common numerical abnormality in these disorders, and one of the most frequent chromosome changes overall.[2] Isolated trisomy 8 displays no gender-related frequency differences in AML or MDS.[72] However, it is more common among elderly AML patients, with a similar trend being present, albeit not statistically significant, in MDS.[72,76]

*Clinical features*
Although +8 is found in all morphologic subgroups of AML and MDS, its frequency has been shown to vary among the different subtypes.[72,77] Previous studies have found the highest incidence of isolated trisomy 8 in the AML M1, M4 and M5 subtypes.[72,77,78] As regards the latter morphology, Haferlach et al.[77] reported that +8 as the sole chromosome aberration was significantly more common in the M5a subgroup (22%) than in M5b (3%). However, a survey of published, unselected cases with trisomy 8 as the sole aberration in relation to different AML subtypes

**Table 1.** Frequency of +8 as the sole aberration in unselected cytogenetically abnormal AML cases reported in the literature[2]

| M0 | M1 | M2 | M3 | M4 | M5 | M5a | M5b | M6 | M7 | Spec | NOS | Total |
|----|----|----|----|----|----|-----|-----|----|----|------|-----|-------|
| 5.2 | 6.1 | 5.8 | 1.8 | 6.8 | 10 | 11 | 11 | 6.3 | 4.4 | 0 | 8.0 | **6.4** |

AML, acute myeloid leukemia; M0 – M7, AML subtypes; Spec, special type; NOS, not otherwise specified.

**Table 2.** Frequency of +8 as the sole aberration in unselected cytogenetically abnormal MDS cases reported in the literature[2]

| RA | RARS | RAEB | CMML | RAEBt | Spec | NOS | Total |
|----|------|------|------|-------|------|-----|-------|
| 10 | 13 | 12 | 14 | 9.6 | 0 | 7.4 | **11** |

MDS, myelodysplastic syndromes; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, RA with excess of blasts; CMML, chronic myelo-monocytic leukemia; RAEBt, RAEB in transformation; Spec, special type; NOS, not otherwise specified.

does not reveal a particularly high frequency of +8 in AML M1 and M4, and identical incidences of isolated +8 are seen in the M5a and M5b subgroups (11%; Table 1). In MDS, +8 has been reported to be most common in chronic myelomonocytic leukemia, refractory anemia, and refractory anemia with excess of blasts,[72,73,75] but no statistically significant differences in the frequency between different morphologic subtypes are detected among published cases (Table 2). Thus, different studies have yielded conflicting results as regards the occurrence of +8 in the various morphologic subgroups, especially in AML. Although these discrepancies may be spurious, reflecting various ascertainment procedures for included cases, it cannot be excluded that, e.g., geographic differences exist in the incidence of trisomy 8 as the sole aberration in AML and MDS. Hence, further studies are needed to clarify this issue.

No specific immunophenotype has been described for AML and MDS with isolated trisomy 8.[79] However, Casasnovas et al.[80] reported that AML cases with +8 mainly expressed CD13 and CD33, and that this cytogenetic subgroup differed from AMLs with abnormal karyotypes by fewer cases expressing CD34.

*Etiology*

Next to nothing is known about environmental risk factors for AML/MDS with +8 as the sole aberration. In fact, trisomy 8 is negatively associated with AML and MDS following iatrogenic exposure, i.e., prior treatment with radio- or chemotherapy; it is significantly more frequent in de novo disease.[72,81] In AML, trisomy 8 is positively associated with previous exposure to organic solvents, in particular benzene.[82] Although the latter compound has been suggested to induce specifically aneuploidy of chromosome 8,[83] more investigations are needed to address this issue. Smoking has also been reported to increase the risk for +8,[84] but this finding could not be confirmed in a later study which included more cases.[85] Taken together, the epidemiologic data are scant, but the fact that +8 is more common among elderly patients[72,76] suggests that prolonged

exposure to one or several as yet unknown environmental factors is involved in the genesis of trisomy 8-positive AML and MDS.

*Constitutional trisomy 8 and myeloid malignancies*
Constitutional trisomy 8, which is almost always associated with mosaicism (CT8M), is a rare condition, estimated to occur in less than 0.1% of all recognized pregnancies.[86] In contrast to other congenital trisomies, such as +21, the nondisjunction event resulting in gain of chromosome 8 in liveborn individuals with CT8M seems to have occurred almost exclusively as a post-zygotic error, and as expected for gains arising through this mechanism, there is no preferential parental origin of the extra chromosome.[87,88] CT8M is associated with mild to moderate mental retardation, characteristic facial dysmorphisms, deep palmar and plantar grooves, bone and joint abnormalities, and cardio-vascular and urogenital malformations.[89] However, the severity of the clinical picture is very variable, and some individuals present with an apparently normal phenotype, including normal intelligence.[89]

       CT8M seems to be associated with an increased risk for neo-plasia, in particular myeloid malignancies.[90-92] Taken together with the fact that some individuals with this mosaicism have a normal phenotype, this has led several investigators to suggest that a subset of AMLs and MDSs with +8 may in fact represent constitutional cases.[90,93-95] In line with this, Maserati et al.[96] reported that two of fourteen trisomy 8-positive myeloid malignancies were previously undetected CT8M. Although isolated +8 in leukemia is hence constitutional in some instances, this rather high frequency (14%) remains to be confirmed in other studies. However, even if the incidence of undetected CT8M in myeloid malignancies proves to be significant, this congenital mosaicism is highly unlikely to be an underlying factor in most cases of AML and MDS with trisomy 8.

*Prognostic impact*
There is some controversy as to the prognostic impact of trisomy 8 as the sole aberration in AML. Although several investigators have included cases with isolated +8 in the intermediate risk group,[71,97,98] others have reported a poor outcome.[70,99-101] In MDS, cases with +8 as the sole change are included in the intermediate risk group,[73-75] although an increased incidence of transformation to AML as compared to other cytogenetic subtypes in MDS has been noted.[73,75] Considering that the intermediate risk groups in AML and MDS are large, additional

prognostic factors are clearly needed to stratify cases with +8 as the sole aberration.

*Cell of origin*
An issue that has received much attention during recent years is the involvement of the hematopoietic stem cell pool in AML and MDS. For AML, the transforming event usually seems to occur in a $CD34^+/CD38^-$ leukemic "stem cell".[102] Such cells have also been shown to harbor trisomy 8 in AML with this abnormality as the sole cytogenetic aberration.[103]

    The hematopoietic cell in which trisomy 8 arises in MDS is more controversial. This is a clinically important question; if the stem cell pool is not involved, autologous stem cell transplantation may become an alternative treatment for this disorder. Early studies, in which morphology-antibody-chromosomes (MAC) techniques were used to identify different cell populations, showed that +8 sometimes was present in all myeloid lineages, including erythroid precursor cells, granulocytes, megakaryocytes, and monocytes, whereas it was not detected in cells of the lymphoid lineages.[104,105] This provided evidence for its occurrence at the colony-forming unit of granulocyte-erythrocyte-macrophage-mega-karyocyte (CFU-GEMM) or pluripotent stem cell level.[104] However, some investigators reported single cases of MDS with clonal involvement of lymphoid cells.[106,107] More recent studies have utilized cell populations obtained by fluorescence-activated cell sorting to analyze the presence of trisomy 8. Thus, Saitoh et al.[108] investigated the occurrence of trisomy 8 – both as the sole aberration and in addition to other changes – in pluripotent stem cells and progenitor cells from seven MDS cases. Trisomy 8 was detected at the level of the CFU-GEMM ($CD34^+CD33^+$), but not in cells of the lymphoid lineages or in hematopoietic stem cells ($CD34^+Thy1^+$). In contrast, Nilsson et al.[109] reported that a variable part of the $CD34^+CD38^-$ (including both $Thy1^-$ and $Thy^+$) cells carried +8, although a sizeable fraction still had disomy for this chromosome. Interestingly, these disomic cells were functionally abnormal, suggesting that they were part of the MDS clone. Furthermore, in four patients with +8 in addition to 5q-, the latter aberration was shown to precede the extra chromosome 8.[109] They concluded that MDS with trisomy 8 seems to involve the hematopoietic stem cell pool, although +8 may not be present initially.[109] Taken together, more studies of trisomy 8-positive MDS are definitely warranted to resolve the important issue of in which cell this chromosome aberration originates.

*Trisomy 8 as a secondary aberration*

In addition to being common as the sole chromosome change in AML and MDS, +8 is a frequent secondary aberration, occurring in association with other abnormalities in approximately 10% of cytogenetically aberrant AML and MDS cases.[2] Secondary +8 is found in all morphologic subtypes of AML and MDS, and is not specifically associated with a certain primary rearrangement,[2,110] although it is particularly frequent in, e.g., AML with t(7;12)(q36;p13) and t(1;11)(p32;q23) and in MDS with der(1;7)(q10;p10) (Tables 3, 4). Schoch et al.[97] reported that trisomy 8 secondary to chromosome abnormalities classified as favorable or poor risk factors in AML did not alter the prognosis based on the primary rearrangements. However, in a subsequent, larger study, Wolman et al.[71] found a significantly worsened outcome in cases assigned to the poor risk group when +8 accompanied the primary abnormalities. Thus, the prognostic impact of trisomy 8 as a secondary change is debated, and more studies are hence needed to determine this clinically important issue.

**Table 3.** Frequency of +8 in addition to well-known primary abnormalities in AML[2]

| Primary change | +8 (%) |
|---|---|
| der(1;7)(q10;p10) | 18 |
| inv(3)(q21q26)/t(3;3)(q21;q26) | 2.1 |
| inv(16)(p13q22)/t(16;16)(p13;p22) | 9.6 |
| t(1;3)(p36;q21) | 0 |
| t(1;11)(p32;q23) | 20 |
| t(1;11)(q21;q23) | 0 |
| t(1;22)(p13;q13) | 0 |
| t(2;11)(p21;q23) | 9.1 |
| t(3;12)(q26;p13) | 0 |
| t(3;21)(q26;q22) | 10 |
| t(4;12)(q11-12;p13) | 5.0 |
| t(6;9)(p23;q34) | 6.2 |
| t(6;11)(q27;q23) | 4.9 |
| t(7;11)(p15;p15) | 5.0 |
| t(7;12)(q36;p13) | 25 |
| t(8;16)(p11;p13) | 5.2 |
| t(8;21)(q22;q22) | 4.9 |
| t(9;11)(p21-22;q23) | 19 |
| t(9;22)(q34;q11) | 16 |
| t(10;11)(p11-13;q23) | 9.7 |
| t(11;17)(q23;q21) | 9.7 |
| t(11;17)(q23;q25) | 11 |
| t(11;19)(q23;p13) | 12 |
| t(15;17)(q22;q11-21) | 12 |
| t(16;21)(p11;q22) | 10 |

AML, acute myeloid leukemia.

**Table 4.** Frequency of +8 in addition to other well-known anomalies in MDS[2]

| Other change | +8 (%) |
| --- | --- |
| del(5q) | 11 |
| del(7q) | 11 |
| del(11q) | 16 |
| del(12p) | 10 |
| del(20q) | 8.2 |
| der(1;7)(q10;p10) | 22 |
| idic(X)(q12-13) | 0 |
| inv(3)(q21q26)/t(3;3)(q21q26) | 0 |
| t(1;3)(p36;q21) | 0 |
| t(3;12)(q26;p13) | 0 |
| t(3;21)(q26;q22) | 5.6 |

MDS, myelodysplastic syndromes.

### *What is the pathogenetic impact of trisomy 8 as the sole aberration in AML and MDS?*

Next to nothing is known about the pathogenetic consequences of +8 in AML and MDS. Possible explanations include global gene deregulation, resulting from the putative gene dosage effects that the duplication of chromosome 8 may confer, deregulation of imprinted loci, or duplication of a cytogenetically cryptic rearrangement/mutation present in the chromosome. However, the latter mechanism is less likely for trisomy 8 in AML and MDS because the occurrence of myeloid malignancies in CT8M cases suggests that additional abnormalities would have to occur after the trisomy.

Is trisomy 8 sufficient for initiating leukemia? The answer to this question is not known. It is possible that additional genetic changes are needed for leukemogenesis; aberrations that would have to be cryptic in AMLs/MDSs with +8 as the sole cytogenetic anomaly. Such hidden changes could be located at chromosome 8, or they could involve other chromosomes. Their identification would be important, not only for the understanding of the biology of trisomy 8-positive AML/MDS, but also clinically, with possible diagnostic, prognostic, and treatment-related ramifications.

### *Pathogenetic impact: Gene dosage effects associated with trisomy 8?*

The pathogenetic impact of +8 has in some instances been considered to equal duplication of the *MYC* oncogene at 8q24, at least in CML.[111,112] However, because chromosome 8 contains approximately 800 genes (the Ensembl Genome Browser; http://www.ensembl.org/), it seems unlikely that the functional outcome of this trisomy is contributable to a single

locus. Furthermore, Mertens et al.[113], by mapping chromosome 8 gains in close to 10,000 cases of myeloid malignant disorders, showed that such imbalances almost always occur in the form of a trisomy and could not be reduced to a single band. Thus, they concluded that the pathogenetic effect of trisomy 8 was unlikely to be upregulation of only one gene on this chromosome, including *MYC*. Also, it has been reported that *MYC* is not overexpressed in trisomy 8-positive AML.[114] In fact, recent studies have shown that *MYC* is not even upregulated in AML/MDS in which it is highly amplified on dmin, suggesting that overexpression of this gene is not a pathogenetic event in these disorders.[115,116] Instead, duplication of chromosome 8 seems to be associated with global changes in the gene expression patterns, as shown in several microarray analyses of AML cases with isolated trisomy 8.[28,114,117,118]

Four microarray studies of trisomy 8 as the sole aberration in AML have been reported. Virtaneva et al.[114] specifically compared cases with +8 to AML with a normal karyotype, whereas the remaining investigations included various additional cytogenetic subgroups. Unsupervised analyses, utilizing all transcripts in the array, did not detect clustering of trisomy 8-positive cases.[114,117,118] However, characteristic gene expression patterns, i.e., clustering, were seen for AML with trisomy 8 in two of three supervised – including only pre-selected genes – analyses.[28,117,118] This suggests that the +8-subgroup has a hetero-geneous gene expression profile compared with AML with well-known primary rearrangements.[117] Furthermore, different genes have been shown to be over- or underexpressed in the investigations. Obviously, this discrepancy could be due to the different array platforms used, but it could also reflect an underlying heterogeneity of trisomy 8-positive AML. A general overexpression of genes on chromosome 8 was noted in three of the analyses, corresponding to 1.27, 1.32, and 1.13, and times the level in AMLs with normal karyotypes, respectively.[28,114,117] However, a substantial proportion of the chromosome 8 genes was not upregulated, demonstrating that gain of one allelic copy does not automatically confer a higher expression of the gene. As regards the biologic function of the differentially expressed genes in trisomy 8-positive AML, Virtaneva et al.[114] found a downregulation of genes involved in apoptosis.

Only one microarray expression study of MDS with isolated +8 has been reported. Chen et al.[119] compared the gene expression profiles of purified CD34-positive cells from patients with trisomy 8 with such cells from monosomy 7 cases. In contrast to the findings in AML, no general overexpression of genes on chromosome 8 was noted in +8-

positive MDSs, although a specific gene expression signature was identified. This included upregulation of genes involved in immune and inflammatory responses.[119]

In summary, microarray expression analyses have shown discrepant results among different AML analyses of trisomy 8 as the sole cytogenetic aberration as well as between such AMLs and MDSs. More studies are needed to determine if these differences are the result of the different experimental procedures, or if a true heterogeneity exists at the gene expression level.

*Pathogenetic impact: Imprinting?*
Although no studies have addressed specifically the parental origin of the gained chromosome 8 in AML and MDS, information is available from a handful of CT8M patients with such disorders and from families with high incidences of AML and MDS. In total, 4 cases with maternal origin of the +8 and two with paternal origin have been reported ($P$ = 0.69),[90,120-122] indicating that no gender-specific duplication occurs. Taken together with the facts that no known chromosome 8 gene has been shown to be imprinted, that no cases of segmental UPD involving chromosome 8 loci have been reported, and that constitutional UPD for chromosome 8 seems to be associated with a normal phenotype,[21,22,123-125] it is unlikely that imprinting effects related to the parental origin of the gained chromosome are of importance in trisomy 8-positive AML and MDS.

*Pathogenetic impact: Is trisomy 8 associated with cryptic changes in AML and MDS?*
Several lines of evidence indicate that cryptic anomalies are present in AML/MDS in which an extra chromosome 8 is the only cytogenetically detectable abnormality. These include: (1) CT8M. Although individuals with this condition have an increased risk of myeloid malignancies, only a minority develop such disorders.[91] Furthermore, AML/MDS do not occur until the affected persons are several years of age,[92,96] strongly suggesting that a latency period, during which additional genetic changes may arise, is necessary for leukemogenesis. (2) The clonal origin of MDS with trisomy 8 as the sole aberration. Although the hematopoietic stem cell pool in +8-positive MDS cases contains a sizable fraction of cells with disomy 8, these cells carry intrinsic deficiencies, indicating that they are part of the malignant clone. Thus, trisomy 8 appears to occur late in MDS and is hence not the primary transformation event.[109] (3) The gene

expression signature. Schoch et al.[28] reported that the discriminating gene expression pattern of AML with isolated trisomy 8 was not dependent on the upregulation of chromosome 8 genes alone, implying the presence of additional genetic changes. (4) +8 as a secondary change. The fact that trisomy 8 is a common secondary aberration in AML and MDS, as well as frequently constituting one of the abnormal clones in polyclonal cases,[2,110,126] suggests that it may be involved in leukemia progression rather than in transformation. (5) Finally, the heterogeneity of +8-positive leukemia. AML/MDS cases with trisomy 8 as the sole cytogenetic aberration differ with regard to clinical factors, morphology, and gene expression patterns.[72,117] This heterogeneity may well be explained by different underlying cryptic genetic changes.

*Studies to identify hidden abnormalities*
Several different FISH investigations have been focused on finding cryptic chromosome aberrations in AML and MDS with trisomy 8 as the sole cytogenetic abnormality. In total, 20 AMLs and MDSs with isolated +8 have been investigated with M-FISH or SKY,[I,127-129] including the 12 cases in article I; only one of these was shown to harbor an additional anomaly. This case was an AML displaying poor G-banding morphology, in which a t(7;14)(q3?1;q2?2) was detected by SKY analysis, but not further characterized.[127] Also, Brown et al.[130] studied one AML with +8 with their subtelomeric multicolor-FISH analysis, utilizing probes for all subtelomeric chromosome regions, but no cryptic abnormality was detected. To address the possibility of structural anomalies within chromosome 8 itself, FISH assays with PCP and subtelomeric probes for 8p and 8q, as well as with gene-specific probes for *FGFR1*, *MYST3* (previously *MOZ*), *RUNX1T1* (previously *ETO*), and *MYC* were performed in the twelve AML/MDS cases included in article I. No hidden aberrations were detected. Finally, Heller et al.[131] used multicolor banding to study chromosome 8 in 11 AMLs/MDSs with this trisomy, of which eight had it as the sole aberration, but all homologues were apparently normal. Thus, various FISH techniques have not yielded any evidence for cytogenetically cryptic rearrangements in the vast majority of +8-positive AML and MDS.

Molecular methods aimed at finding additional abnormalities include Southern blot, reverse transcriptase-PCR (RT-PCR), and single nucleotide polymorphism (SNP) arrays. For example, Diaz et al.[132] used the first of these techniques to investigate the *MYC* and *MOS* genes in 6 MDS cases with isolated trisomy 8 and 4 AMLs with +8 in addition to

other changes, whereas Langabeer et al.[133], recognizing that +8 is a common secondary change in AML with t(15;17) (Table 3), analyzed 54 trisomy 8-positive AMLs for the presence of cryptic *PML/RARA* fusions with RT-PCR. Also, the possibility of partial UPD was addressed by Raghavan et al.[21] in two AMLs with trisomy 8. Apart from one MDS showing a rearranged *Hin*dIII fragment for *MYC* − not further investigated − in the study by Diaz et al.[132], none of these studies identified any hidden abnormalities.

Studies of the presence of somatic point mutations of leukemia-associated genes have proved somewhat more fruitful. Thus, several AMLs/MDSs with +8 as the sole cytogenetic aberration have been reported to harbor, e.g., *CEBPA*, *FLT3*, *KRAS*, *NRAS*, and *RUNX1* mutations.[134-137] However, none of these abnormalities have been specifically associated with, or particularly frequent in, +8 cases.

Finally, array CGH analysis has been used to investigate 10 AMLs/MDSs with trisomy 8 as the sole cytogenetic aberration.[II] Interestingly, this assay detected cryptic intra-chromosomal imbalances, not corresponding to known CNPs in the human genome, in 5 of the 10 cases (in 1 MDS and 4 AMLs). These changes, some of which were confirmed by FISH, included both segmental duplications and hemizygous deletions and involved several different chromosomes, although not chromosome 8. Most notably, at least two of the identified imbalances were almost certainly leukemia-associated: a del(7)(p14p14), shown to have occurred before the trisomy 8, and a hemizygous deletion of the region surrounding *ETV6* in 12p13; an aberration which previously has been described in AML. Hence, this study indicated that additional cryptic chromosome abnormalities may indeed be present at a high frequency in AML and MDS with +8 as the sole cytogenetic aberration.

## *Conclusions and future directions*

What are the functional outcome and the pathogenetic effect of trisomy 8 as the sole aberration in AML and MDS? Recent microarray studies suggest that +8 is associated with specific gene expression patterns, at least partly associated with dosage effects of chromosome 8 genes.[28,114,117] It is conceivable that such a deregulation is associated with leukemia, and this may well be the direct biologic effect of the trisomy. However, the above-mentioned possibility of cryptic additional anomalies in myeloid malignancies with isolated trisomy 8 is an issue of great clinical and pathogenetic importance. In this context, the findings described in article II are important. Although none of the identified

aberrations in that study were obvious primary, leukemia-inducing anomalies, their identification nevertheless provided the first solid evidence for a high frequency of additional chromosome abnormalities in AML/MDS with trisomy 8 as the sole cytogenetic aberration. Future investigations using array-based CGH or similar methods will determine whether the detected incidence – 4/6 (67%) AMLs and 1/4 (25%) MDSs – of cases having hidden intra-chromosomal imbalances is representative for this cytogenetic subgroup. Furthermore, although rearrangements involving large chromosome parts can be excluded to be common in AML/MDS with isolated +8, based on previous FISH investigations,[I,127-129] intra-chromosomal rearrangements and translocations involving subtelomeric parts remain a possibility. Also, the recent findings of partial UPD in AML cases with other abnormalities is intriguing,[21] warranting further studies of AMLs and MDSs with trisomy 8 as the seemingly sole anomaly. In summary, many important issues remain to be addressed regarding this frequent chromosome aberration.

## HYPERDIPLOIDY IN CHILDHOOD ALL

High hyperdiploidy, i.e., more than 50 chromosomes, is the most common cytogenetic pattern in childhood ALL.[2] Hence, the elucidation of its genetic and clinical features, origin, and pathogenetic effects is of great importance. Below, the present knowledge of hyperdiploid childhood ALL is summarized, including the results presented in articles III and IV.

### Cytogenetic findings

The hyperdiploid cytogenetic subgroup in childhood ALL is characterized by the presence of extra chromosomes, resulting in modal numbers of >50 chromosomes. The gains are nonrandom, and typically involve chromosomes X, 4, 6, 8, 10, 14, 17, 18, and 21. Chromosome 21 is frequently tetrasomic and sometimes pentasomic, whereas the other imbalances are usually found in the form of trisomies, although occasional tetrasomies are seen. Not all tri-/tetrasomies are equally common: gain of chromosome 21 is seen in virtually all hyperdiploid pediatric ALLs, whereas the remaining imbalances are present in 60 – 90%, except +8, which occurs with a frequency of 40% (Table 5).

The G-banding morphology is often poor in the hyperdiploid subgroup; thus, misclassification of chromosomes is quite common. A

comparison between the frequencies of gained chromosomes in cyto-
genetic and FISH investigations shows similar incidences, but with a
trend towards higher rates of most trisomies/tetrasomies in cases
analyzed with FISH (Table 5). Also, the G-banding karyotypes reported
by Moorman et al.[139] – including more than half of the cytogenetically
investigated cases in Table 5 – were reviewed centrally, with cases
displaying incomplete karyotypes being excluded; G-banding results in
general may be expected to be less accurate. Thus, it seems safe to
conclude that FISH analyses are usually superior for identification of
gained chromosomes in hyperdiploid cases.

In addition to the tri- and tetrasomies, G-banding analysis of
hyperdiploid childhood ALL reveals structural changes in approximately
half of the cases (Table 5). Although such abnormalities may go
undetected due to poor chromosome morphology, this frequency is not
increased when cases are investigated with SKY or M-FISH (Table 5).
The most common structural change in hyperdiploid childhood ALL is
duplication of 1q, which is present in 8 – 10% of the cases, followed by
other 1q abnormalities (6%), deletions involving 6q (5 – 7%), and iso-
chromosome 17q (2 – 5%).[2,139] Translocations are seen in 20% of the
cases, including a small subset of cases with well-known primary ALL
rearrangements such as t(1;19)(q23;p13) and t(9;22)(q34;q11).[140]

Hyperdiploid childhood ALL is generally karyotypically stable,
i.e., these cases display little or no cell-to-cell variation when
investigated with cytogenetic techniques. Teixeira and Heim[145] found
evidence for clonal evolution in only 5% of the cases in a review of all
published hyperdiploid ALLs. However, because of the poor chromo-
some morphology in many of the reviewed studies, the actual frequency
may be higher.[145] Approximately 20% of the cases reported by Raimondi
et al.[140] harbored subclones, which usually differed from the major clone
by two to three additional chromosomes or structural rearrangements.
This is in line with the frequencies detected by SKY/M-FISH; two such
studies have reported cytogenetic subclones in 2/18 (11%)[142] and 8/27
(30%)[IV] cases, respectively. However, it should be stressed that hyper-
diploid childhood ALL does not display the general CIN seen in many
aneuploid solid tumors.

*Epidemiology and etiology*
Hyperdiploidy has been reported to occur in 20 – 30% of all pediatric
ALL cases, making it the largest cytogenetic subgroup.[26,139,146-149] It has
been suggested that the  frequencies of hyperdiploid childhood ALL  vary

**Table 5.** Comparison between chromosome aberrations detected in G-banding and FISH analyses of hyperdiploid childhood ALL[a]

| Chromosome aberration | Cytogenetic investigations (% of the cases) | | | | FISH investigations[b] (% of the cases) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Raimondi et al.[140] (n=182) | Heerema et al.[141] (n=480) | Moorman et al.[139] (n=700) | Mean | Moorman et al.[138] (n=146) | Elghezal et al.[142] (n=18) | Nordgren et al.[143] (n=15) | Nordgren et al.[144] (n=13) | Paulsson et al.[IV] (n=27) | Mean |
| Gain of X | 81 | 88 | 89 | **86** | 95 | 100 | 100 | 100 | 81 | **95** |
| Gain of 4 | 76 | 74 | 78 | **76** | 75 | 67 | 60 | 54 | 74 | **66** |
| Gain of 6 | 86 | 85 | 85 | **85** | 86 | 78 | 87 | 69 | 81 | **80** |
| Gain of 8 | 34 | 37 | - | **36** | - | 39 | 33 | 31 | 52 | **39** |
| Gain of 10 | 56 | 63 | 63 | **61** | 61 | 78 | 47 | 54 | 74 | **63** |
| Gain of 14 | 80 | 81 | 84 | **82** | 90 | 100 | 93 | 100 | 93 | **95** |
| Gain of 17 | 68 | 67 | 68 | **68** | 65 | 67 | 93 | 92 | 70 | **77** |
| Gain of 18 | 68 | 73 | 76 | **72** | 78 | 78 | 73 | 54 | 81 | **73** |
| Gain of 21 | 97 | 98 | 99 | **98** | 100 | 100 | 100 | 100 | 100 | **100** |
| Tetrasomy 21[c] | 66 | 69 | 70 | **68** | - | 83 | 53 | 77 | 85 | **75** |
| Structural changes | 54 | - | 53 | **54** | - | 39 | 33 | 38 | 50[d] | **40** |

FISH, fluorescence in situ hybridization; ALL, acute lymphoblastic leukemia; SKY, spectral karyotyping; M-FISH, multicolor-FISH.
[a]Including cases with 51–65 chromosomes, except for Raimondi et al.[140] (51–67 chromosomes) and Heerema et al.[141] (>50 chromosomes).
[b]Analyzed with SKY, except for Moorman et al.[138] (analyzed with interphase FISH using probes for chromosomes X, 4, 6, 10, 14, 17, 18, and 21) and Paulsson et al.[IV] (analyzed with M-FISH in ten cases and with interphase FISH using probes for chromosomes X, 4, 6, 8, 10, 14, 17, 18, and 21 in seventeen cases).
[c]May include occasional cases with pentasomy 21.
[d]% of the ten cases investigated with M-FISH.

among different populations; for example, a higher incidence has been noted in the Nordic countries.[26,146,150] Hyperdiploidy is strongly associated with young age, being most common in children below the age of ten, with a median age of 4 years at diagnosis.[140,147,148,151,152] There is no gender-related frequency difference.[140,148]

 Little is known about environmental risk factors for hyperdiploid childhood ALL. As described below, this cytogenetic feature appears to arise already in utero,[153-157] suggesting that exposure to transforming agents during pregnancy may be of importance. Wiemels et al.[158] reported that hyperdiploidy is significantly less common in children prenatally exposed to paternal smoking. They hence speculated that smoking-associated mutagens may be toxic to hyperdiploid clones,[158] but further studies are needed to clarify this issue. Also, Hjalgrim et al.[159] reported that an elevated birth weight increased the risk for hyperdiploid childhood ALL. However, this risk factor seemed to be associated with pediatric ALL in general and not specifically with the hyperdiploid subtype.[159]

 An increased risk for childhood ALL, albeit not specifically for the hyperdiploid subtype, has been associated with low folate intake during pregnancy.[160] Interestingly, folate deficiency has been shown to induce aneuploidy of chromosomes 17 and 21 in cultured lymphocytes, suggesting a possible association between disturbances in this metabolic pathway and hyperdiploidy.[161] Furthermore, results by Wiemels et al.[162] indicated that constitutional homozygosity for polymorphisms in the *MTHFR* gene, encoding an enzyme active in the folate pathway, may be less common among hyperdiploid childhood ALL cases. Taken together, the possibility of an association between folate metabolism and hyperdiploidy is interesting, warranting further investigations.

*Clinical features*

Hyperdiploidy in childhood ALL is associated with favorable prognostic factors, including low WBC ($<10 \times 10^9/l$) and age between 1 to 9 years.[140,146-149,151,152] Involvement of the central nervous system is seen in less than 5% of the cases and the presence of a mediastinal mass is rare.[140,141,148,152,163,164] Furthermore, hyperdiploidy is associated with an early pre-B immunophenotype,[151] typically $CD34^+CD10^+CD19^+CD45^-$ $CD22^+CD24^+TdT^+IgM^-CD66c^+$.[79]

*Prognostic impact*

The hyperdiploid cytogenetic subgroup is associated with a favorable outcome in childhood ALL, first described by Lampert[165] as early as in 1967 and confirmed in later studies.[147,149,163,166-169] Thus, patients within this karyotypic subgroup have an overall survival of approximately 90%.[139]

Several studies have addressed the possibility that specific trisomies may have a prognostic impact. In the first of these, Jackson et al.[170] found that +6 was associated with a favorable prognosis; however, that investigation also included cases with 47 – 50 chromosomes, and was hence not restricted to the hyperdiploid subgroup. Subsequently, Harris et al.[171], analyzing 1,000 children with pre-B ALL, detected increased event-free survival for cases with +4 and +10; an association that was not seen in a later study of 182 hyperdiploid ALLs by Raimondi et al.[140] Heerema et al.[141] concluded, in an investigation of 480 ALLs with >50 chromosomes, that whereas +4 and +6 did not affect the prognosis, concurrent +10 and +17 were associated with a superior outcome and +5 conferred a poor prognosis. Furthermore, Moorman et al.[139] reported an association between improved outcome and trisomies 4 and 18 among 700 cases of hyperdiploid childhood ALL, and Sutcliffe et al.[172], investigating +4, +10, and +17 in a large study of >5,000 hyperdiploid cases, found that these trisomies were associated with a favorable prognosis. Although these results seem conflicting, most studies have detected specific effects of trisomies 4, 10, 17, and 18 in univariate analyses, but the conclusions drawn from the multivariate analyses have differed.[139] Thus, it seems likely that certain trisomies might affect the prognosis in hyperdiploid childhood ALL, but more investigations are needed to clarify this clinically important issue.

It has also been suggested that the modal number may be prognostically important in hyperdiploid cases. Thus, patients with >53 or >55 chromosomes have been reported to fare better than those with 51 – 53/55 chromosomes.[139-141] However, a recent study have shown that this effect cannot be separated from the above-mentioned impact of specific trisomies.[139]

Pui et al.[164] reported an inferior outcome for cases with structural chromosome changes in addition to the hyperdiploid karyotype. This has not, however, been confirmed in subsequent, larger studies, with the exception of the rare hyperdiploid cases carrying t(1;19), t(8;14), t(9;22), or translocations involving 11q23.[139,140,163] Specifically, isochromosome 17q has been suggested to be an adverse

cytogenetic feature in hyperdiploid childhood ALL, but in recent investigations, no such effect has been seen.[139,140,163,173]

What is the reason for the favorable outcome of hyperdiploid childhood ALLs as compared to other cases? Several investigators have shown that the blast cells from these ALLs are particularly sensitive to chemotherapy, especially to methotrexate (MTX).[174-178] This sensitivity may be associated with gains of chromosome 21, because the *SLC19A1* (previously *RFC1*) gene, encoding a protein that transports MTX into the cell, is located at 21q22.[177-179] Trisomy/tetrasomy 21 has been shown to upregulate *SLC19A1*, presumably resulting in higher concentrations of MTX in hyperdiploid blast cells.[177,178] Furthermore, it has been shown that hyperdiploid cells have an increased propensity to undergo apoptosis, both in vitro in stromal cultures and in vivo in bone marrows.[180-182] Thus, the superior prognosis of this cytogenetic subgroup in childhood ALL may be due to a combination of high sensitivity to chemotherapy in association with an increased incidence of spontaneous apoptosis.

*Cell of origin*

Larramendy et al.[183] investigated five cases of hyperdiploid childhood ALL by the MAC technique and found that the extra chromosomes were restricted to cells of the lymphoid lineages in all but one of the cases. The latter ALL displayed hyperdiploidy in a subset of the erythroid cells, indicating that this cytogenetic feature may have arisen in a pluripotent hematopoietic stem cell.[183] However, Kasprzyk et al.[184], investigating B-lineage (CD19^{+}), T-lineage (CD3^{+}), myeloid (CD13^{+}), and erythroid (glycophorin A^{+}) cells in nine cases, detected hyperdiploidy only in the first of these groups. They hence suggested that the chromosome gain arose in a lymphoid committed progenitor cell, although occurrence at the pluripotent stem cell level could not be excluded.[184] Quijano et al.[185] reported that hyperdiploidy was present in a subset of the CD34^{+}CD33^{-}CD38^{-}CD19^{-} compartment – i.e., in CD34^{+}, but lineage negative, cells – in 3/12 investigated cases, thus indicating the latter. Taken together, the analyses performed so far suggest that hyperdiploidy arises in a lineage uncommitted stem cell, possibly without the ability to form myeloid cells.[184] However, this issue is not yet definitively clarified.

*Hyperdiploidy arises in utero*

That some childhood leukemias may arise already in utero was first suggested by reports of twins with concordant ALL,[186] and later directly

demonstrated by Gale et al.[187], who showed that the *MLL/AFF1* (previously *AF4*) fusion transcript, resulting from the t(4;11)(q21;q23), was present in neonatal blood spots (Guthrie cards) from patients who later developed ALL with this translocation. Subsequently, several cytogenetic abnormalities associated with childhood leukemia have been shown to occur before birth.[186]

The first indication that hyperdiploidy may be included in this group came from an investigation by Yagi et al.[153], who screened neonatal blood spots from patients who later developed ALL for the presence of the clonotypic somatic recombination of *IGH@*, the heavy immunoglobulin gene at 14q32. One of the positive cases was a hyperdiploid ALL, diagnosed when the patient was two years old.[153] Similar results were subsequently reported by Taub et al.[154], who found clonotypic *IGH@* rearrangements in 6/6 Guthrie cards from patients with such ALL. However, from these findings, it could not be confirmed that the detected clones harbored hyperdiploidy at the time of birth; this cytogenetic feature could have arisen later.[154]

Szczpansky et al.[188] reported that ALL with trisomy 14 sometimes displayed three different *IGH@* rearrangements, but they did not investigate the possibility of a prenatal event. Instead, this was done by Panzer-Grümayer et al.[155], who detected three clonotypic *IGH@* rearrangements, all derived from a single abnormal clone, in the Guthrie card from one patient with hyperdiploid, +14-positive ALL. Thus, they provided the first solid evidence for a prenatal origin of hyperdiploidy – or at least for trisomy 14 – albeit in a single case. Subsequently, a pair of monozygotic twins with concordant hyperdiploid ALL, having identical chromosome aberrations and shared *TRDJ1/TRDD3* (previously *TCRD*) and *IGH@* rearrangements, was reported by Maia et al.[156], again demonstrating that the hyperdiploidy arose in utero. More recently, the presence of +15 and +17 was demonstrated in $CD34^+CD19^+$ cord blood cells from a child who later developed a hyperdiploid ALL with these trisomies.[157] Taken together, these findings show that hyperdiploidy is a prenatal event in at least some cases of childhood ALL, and provide strong support for the possibility that all such cases arise in utero.

*How does hyperdiploidy arise?*
As described above, hyperdiploidy in pediatric ALL is probably acquired already in utero, in an immature B-lineage cell. How, then, do these multiple gains of chromosomes happen? Theoretically, hyperdiploidy may arise by four different mechanisms: (1) by initial near-haploidy
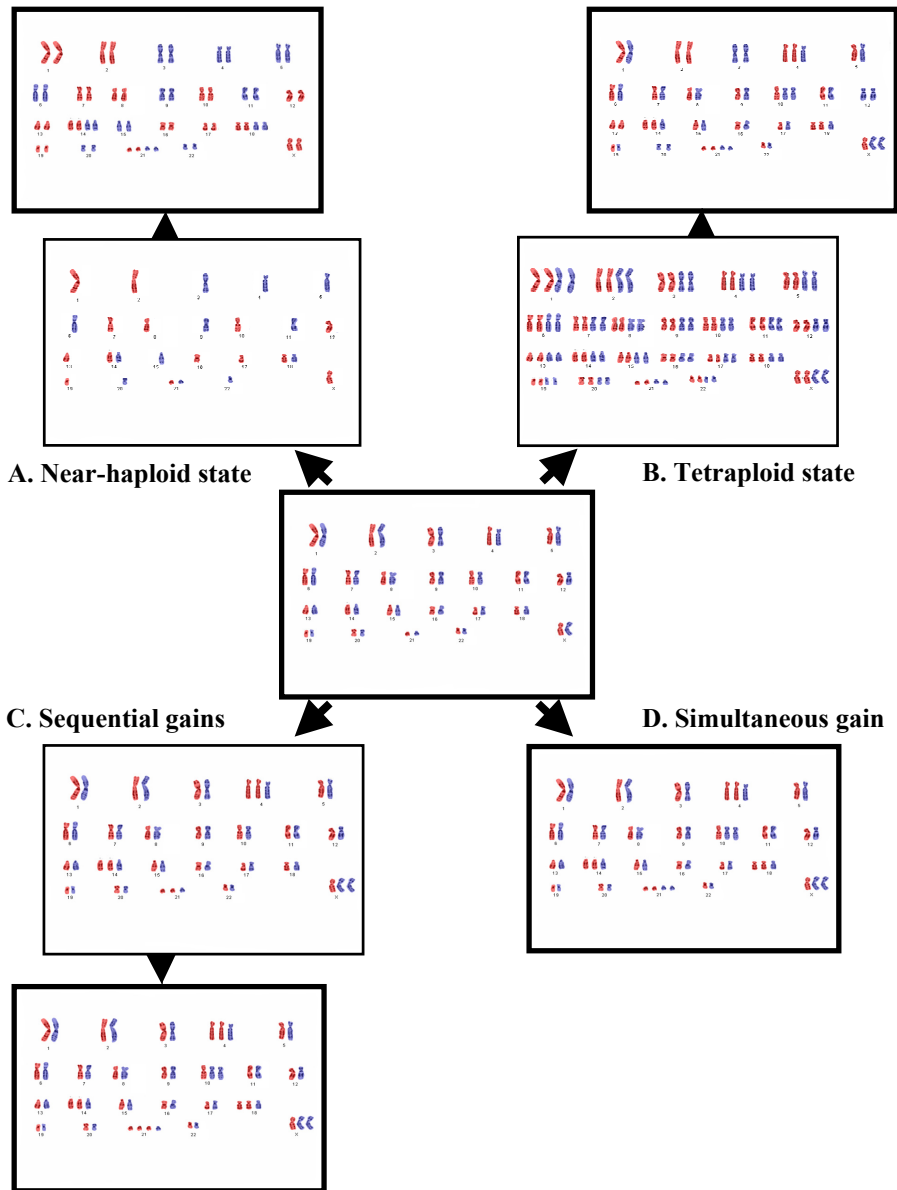
**Figure 3.** Four possible mechanisms for the formation of hyperdiploidy. UPD, uniparental disomy; EAD, equal allele dosage; UAD, unequal allele dosage. (A) Doubling of a near-haploid set of chromosomes, resulting in UPDs for all disomic chromosomes and EAD for tetrasomies. (B) Initial tetraploidization with subsequent losses of chromosomes, resulting in UPDs for approximately one third of the disomic chromosomes and EAD for tetrasomies. (C) Sequential gains of individual chromosomes in consecutive cell divisions, resulting in no UPDs and UAD for two thirds of the tetrasomies. (D) Simultaneous gain of chromosomes in a single abnormal mitosis, resulting in no UPDs and EAD for all tetrasomies.

followed by doubling of the chromosomes, (2) by tetraploidization with subsequent chromosome losses, (3) by sequential gains of chromosomes in consecutive cell divisions, or (4) by a simultaneous gain of chromosomes in a single abnormal mitosis (Fig. 3).[189] Considering that we cannot directly observe the transformation event, the evidence for and against these mechanisms is indirect. However, investigations of the allele dosage for genes in tetrasomic chromosomes, as well as of the presence of UPDs, may provide valuable clues (Fig. 4). In total, three studies addressing the mechanism behind hyperdiploidy have been published. These include one investigation by Onodera et al.[189] of 15 hyperdiploid childhood ALLs using restriction fragment length polymorphisms and articles III and IV, utilizing QF-PCR with polymorphic microsatellite markers to study 10 and 27 cases, respectively.

　　　　Starting with the tetrasomies, these may display either equal (EAD) or unequal (UAD) allele dosage (Fig. 4A). The former is a 2:2 allele ratio for all loci at that chromosome, resulting from duplication of both homologues, whereas the latter is a 3:1 ratio for all loci, because of triplication of one homologue and retention of the other (Fig. 4A). Experimental evidence indicates that EAD for tetrasomies is an almost universal feature of hyperdiploid childhood ALL.[189,III,IV] Thus, tetrasomy 21, which is the most common tetrasomy and also the most well-investigated, has been shown to display EAD in 31 of 33 (94%) cases.[189,III,IV] It should be noted that the +21,+21 in the two cases that had UAD were identified with G-banding only and it is hence possible that they had an unrecognized pentasomy for chromosome 21. The only study that included FISH to confirm all gains – article IV – did not detect any cases with unequal allele dosage for +21,+21. As regards other tetrasomies, fifteen such changes were investigated in article IV, involving chromosomes X, 8, 10, 14, and 18. Thirteen (87%) displayed equal allele dosage. The two exceptions – +8,+8 and +14,+14 – were present in the same case.[IV] It should also be mentioned that Haas et al.[190] investigated seven ALLs with tetrasomy X by a FISH X inactivation assay; all had duplicated both the active and the inactive X, i.e., the equivalent of EAD. Thus, of the altogether 55 tetrasomies that have been analyzed in hyperdiploid childhood ALL cases, 51 (93%) have displayed duplication of both homologues, as opposed to triplication of one homologue (Fig. 4A).[189,190,III,IV]

　　　　Clues to the mechanism behind hyperdiploidy from disomic chromosomes are given by the possible occurrence of UPDs, i.e., both

## A. Tetrasomies



**Retained disomy**

*Disomy*

*Tetrasomy*

**EAD**

*Tetrasomy*

**UAD**

## B. Disomies



*Disomy*

*UPD*

**Retained disomy**

**LOH**
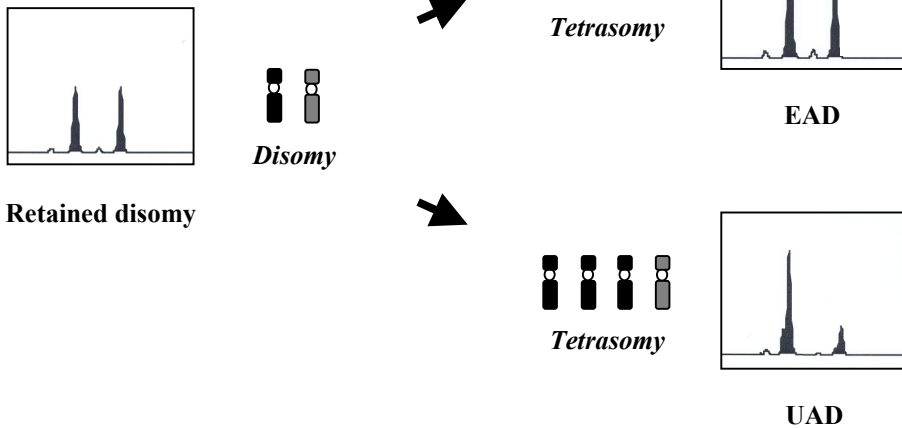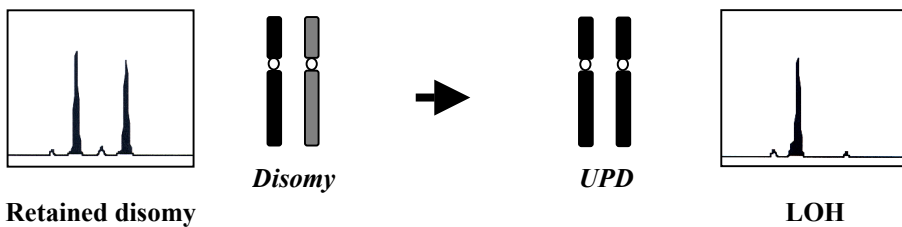
**Figure 4.** QF-PCR findings for aberrant chromosomes. Representative electrophero-grams are shown next to chromosomes. Each peak represents an allele for a poly-morphic microsatellite marker. EAD, equal allele dosage; UAD, unequal allele dosage; UPD, uniparental disomy; LOH, loss of heterozygosity. (A) Tetrasomies may result either from the duplication of both homologues or from the triplication of one homologue. The former leads to EAD, visible as two peaks with a 2:2 ratio. The latter results in UAD, visible as two peaks with a 3:1 ratio. (B) UPD – both homologues being derived from the same parental chromosome – is visible as LOH, i.e., complete loss of one peak.

homologues being derived from a single parental chromosome (Fig. 4B). This is detectable as loss of heterozygosity (LOH) for all loci at that chromosome pair (Fig. 4B). In the study by Onodera et al.[189] and in articles III and IV, a subset of all disomic chromosomes was investigated for the presence of UPD. Apart from three cases, further discussed

below, in which UPDs were seen for all disomic chromosomes, only a handful of UPDs were detected among the 52 cases included in these three studies.[189,III,IV] Furthermore, in article IV, a systematic investigation of all disomic chromosomes in ten cases was performed, revealing one case with UPDs for 4/9 disomies, one with UPDs for 2/14 disomies, and one with UPD for 1/10 disomies. Finally, Irving et al.[191] studied all chromosomes in three cases of hyperdiploid childhood ALL with SNP arrays. They detected UPDs for 4/19 disomic chromosomes in one case.[191] Taken together, hyperdiploid childhood ALLs display different frequencies of UPDs, with the majority of the cases having no UPDs, a subset harboring a few UPDs, and a subset displaying UPDs for all disomic chromosomes.[189,191,III,IV]

*The mechanism behind hyperdiploidy*
The first of the above-mentioned mechanisms for formation of a hyperdiploid karyotype involves a near-haploid step (Fig. 3A). Near-haploidy of 23 – 29 chromosomes is a rare but recurrent cytogenetic feature of childhood ALL, occurring in less than 1% of the cases.[2,192] It is characterized by a specific chromosome pattern, with most cases retaining disomies 21, X, Y, 14, and 18, and having monosomies for the remaining chromosomes.[192] This cytogenetic subgroup is associated with a poor prognosis.[192] Already in 1977, Oshimura et al.[193] reported a case of near-haploid ALL with 27 chromosomes that harbored a second clone containing an exact duplicate of the chromosomes present in the first. Subsequent investigations have shown that such related clones are common in the near-haploid subgroup of childhood ALL; e.g., in 9/14 (64%) of the cases reported by Harrison et al.[192] This raised the possibility that hyperdiploid cases may have originated as near-haploid, with subsequent duplication of chromosomes and loss of the original stem line, at least at the cytogenetic level.[194]

By molecular genetic techniques, hyperdiploidy arising via a near-haploid state would be detectable by having UPDs for all disomic chromosomes and EAD for all tetrasomic chromosomes (Fig. 3A).[189] The first experimental evidence for occurrence of this mechanism was presented by Stamberg et al.[195], who described a hyperdiploid childhood ALL characterized by tetrasomies only and with a reduction from heterozygosity to homozygosity for a chromosome 15 polymorphism. Subsequently, Onodera et al.[196] reported that all loci at disomic chromosomes displayed LOH, indicative of UPDs, in two hyperdiploid cases; a similar case is also described in article IV. Furthermore, FISH studies of

apparently hyperdiploid cases have revealed near-haploid clones in inter-phase cells in at least four cases.[197,198] Hence, a subset of hyperdiploid childhood ALLs originates through near-haploidy.

Does the above mechanism explain all hyperdiploid cases in this disorder? The short answer to that question is "no". As described above, molecular studies performed by Onodera et al.[189] and Irving et al.[191] and those presented in articles III and IV have shown that the vast majority of disomic chromosomes in hyperdiploid cases display retained heterozygosity, excluding a near-haploid pathway. Only three of a total of 55 investigated cases had UPD for all disomies.[189,III,IV] Thus, most hyperdiploid cases must be formed by another mechanism. It should also be stressed that the few cases that arise via a near-haploid state should probably be assigned to the near-haploid cytogenetic subgroup and not associated with the "true" hyperdiploid cases. Considering the inferior prognosis of near-haploid pediatric ALL,[192] it is extremely important to identify these cases, e.g., by being observant for hyperdiploid ALLs displaying only tetrasomies and no trisomies.

This leaves three possible mechanisms for the occurrence of "true" hyperdiploidy in childhood ALL (Fig. 3B – D). In the first of these alternatives, the cell initially becomes tetraploid and then loses chromosomes until it is hyperdiploid (Fig. 3B). Hyperdiploidy formed via a tetraploid state would display EAD for all tetrasomies and UPDs for approximately one third of the disomic chromosomes (Fig. 3B). As mentioned above, almost all tetrasomic chromosomes in hyperdiploid childhood ALL display EAD,[189,190,III,IV] in agreement with what be expected for this pathway. UPDs are less common.[189,191,III,IV] However, in studies including all disomic chromosomes, a subset of cases has been shown to harbor some UPDs.[191,IV] Thus, in article IV and the study by Irving et al.[199], this mechanism could not be excluded in 3/10 and 1/3 cases, respectively. Taken together, it is possible that some cases of hyperdiploidy arise by initial tetraploidization with subsequent losses of chromosomes.

Another putative pathway to hyperdiploidy is by sequential gains of chromosomes in consecutive mitoses (Fig. 3C). This is an attractive possibility, because it would fit well with our current view of cytogenetic clonal evolution in malignancies. However, it is not supported by findings at the cytogenetic level, i.e., hyperdiploid ALL rarely harbor subclones when investigated with G-banding.[140,145] At the molecular level, hyperdiploidy formed by this pathway is expected to display UAD for approximately two thirds of the tetrasomic chromo-

somes and no UPDs (Fig. 3C). Although the latter agrees well with the findings in the majority of the analyzed cases, the former does not; UAD has only been detected for 7% of tetrasomies in hyperdiploid childhood ALL, and not all of these chromosomes have actually been confirmed to be tetrasomic by FISH.[189-191,III,IV] Thus, this mechanism is highly unlikely for most cases. However, a possible exception exists; the one case described in article IV in which 2/2 tetrasomies displayed UAD. In this single case, formation by sequential gains of chromosomes may have occurred.[IV]

The final possible pathway for formation of hyperdiploidy is by a simultaneous gain of all extra chromosomes in a single abnormal cell division (Fig. 3D). Such cases would display EAD for all tetrasomies and no UPDs. This pattern agrees well with most molecularly investigated hyperdiploid childhood ALLs; 51/55 (93%) tetrasomic chromosomes have shown EAD and the vast majority of the disomies have not been UPDs.[189-191,III,IV] Hence, it is likely that most cases of hyperdiploidy in childhood ALL arise through a simultaneous gain of chromosomes.[189,III,IV]

Taken together, 55 cases of hyperdiploid childhood ALL have been sufficiently analyzed with molecular techniques to determine by which pathway they were formed. Three of these cases (5%) displayed all the expected features of hyperdiploidy arising via a near-haploid state. Four cases (7%) may have arisen through a tetraploid state, although sequential gains or a simultaneous gain are also possible in at least two of these. One case (2%) most likely originated by a sequential gain of chromosomes. Seven cases (13%) were shown to have arisen by a simultaneous gain of chromosomes. Finally, the remaining 40 cases (77%) displayed patterns of EAD/UAD and UPDs strongly suggesting a simultaneous gain. However, formation via a tetraploid state or by sequential gains could not be definitely excluded, although they were highly unlikely based on the combined molecular findings. Thus, different childhood ALL cases may have hyperdiploidy originating by different mechanisms. This may be clinically important – for example, the prognosis for hyperdiploid cases arising via a tetraploid state or by sequential gains could possibly be inferior to that of cases occurring through a simultaneous gain of chromosomes in a single abnormal mitosis. Studies of clinical features associated with hyperdiploidy originating by different pathways are therefore warranted.

*What is the pathogenetic impact of hyperdiploidy?*

Like for other trisomies in hematologic malignancies, little is known about the biologic importance of hyperdiploidy in leukemogenesis. Possible pathogenetic effects include duplication of mutated loci on the gained chromosomes, deregulation of imprinted loci, and gene dosage effects. Furthermore, additional genetic alterations besides the hyperdiploidy may be needed for the development of ALL.

*Hyperdiploidy – sufficient for leukemogenesis?*

As described in detail above, several lines of evidence – including clonotypic *IGH@* rearrangements, concordant hyperdiploid ALL in twins, and the presence of hyperdiploid cells in cord blood – strongly suggest that hyperdiploidy frequently, possibly always, arises before birth.[153-157] However, the twelve patients in whom a prenatal origin has been demonstrated have had a median age at diagnosis of 2.4 years, with the oldest being 9 years old.[153-157] Also, the incidence of hyperdiploidy in childhood ALL is highest in the 1 – 9 years age group.[140,148,151] This strongly suggests that hyperdiploidy is not sufficient for leukemogenesis; additional genetic aberrations are needed for leukemia to develop.

What are these changes? Structural abnormalities are present in approximately half of all hyperdiploid childhood ALLs (Table 5). The most common of these are unbalanced, such as duplication of 1q, deletions of 6q, and isochromosome 17q, and although they may certainly be important in the leukemogenic process, none of them are present in more than a few percent of the cases.[2,139] Within the hyperdiploid subgroup, structural aberrations have been reported to be associated with an increased age,[148] possibly suggesting a different etiology in these cases. However, cytogenetic as well as M-FISH and SKY analyses have failed to detect any primary fusion gene-forming rearrangements in the majority of hyperdiploid ALLs.[139,140,142-144,IV] In contrast, mutations and deregulation of specific, leukemia-associated genes have been shown to occur with high frequencies in this cytogenetic subgroup. Thus, Taketani et al.[200] and Armstrong et al.[201] demonstrated that activating point mutations in the *FLT3* gene are present in 20 – 25% of all cases with hyperdiploid childhood ALL, Tartaglia et al.[202] found an association between *PTPN11* mutations and this karyotypic feature, Wiemels et al.[158] detected mutations in the related *NRAS* and *KRAS* genes at a frequency of 30%, and Zheng et al.[203] found that the promoter region of the putative tumor suppressor gene *FHIT* was hypermethylated, resulting in its inactivation, in more than half of the hyperdiploid cases. It

is noteworthy that the *FLT3*, *PTPN11*, *NRAS* and *KRAS* genes are all involved in the *RAS* signaling pathways, indicating that such deregulation may be a complementary event to hyperdiploidy in childhood ALL.[158] However, other genetic changes may also be required for leukemogenesis.

*Pathogenetic impact – duplicated loci?*

Some acquired trisomies in neoplasias have been shown to be associated with duplication of a mutated gene.[40-43] However, this is unlikely to be the pathogenetic basis for hyperdiploidy, because mutated alleles would have to be present at each gained chromosome. Furthermore, the fact that the overwhelming majority of tetrasomies in hyperdiploid childhood ALL display EAD, i.e., duplication of both parental homologues,[189,190,III,IV] strongly argues against duplication of a specific chromosome. Also, because hyperdiploidy generally arises prenatally,[153-157] possible mutations would have to occur in utero, leaving little time for the, in this scenario, secondary gain of chromosomes. Hence, it seems highly unlikely that hyperdiploidy is generally associated with duplication of mutated genes.

*Pathogenetic impact – imprinting?*

That imprinting effects may be of importance in hyperdiploid childhood ALL was suggested by Haas[48], who speculated that gains of maternally or paternally derived chromosomes could result in deregulation of imprinted loci. This would be detectable as a skewed parental origin for the extra chromosomes.[48] To address this issue, ten hyperdiploid cases were investigated with polymorphic microsatellite markers for all commonly tri-/tetrasomic chromosomes in article III. None of the analyzed chromosomes displayed preferential duplication contributable to the parental origin, arguing against imprinting effects. However, trends towards significance were seen for chromosomes 8 and 14: 4/4 cases with +8 had gained the paternally inherited homologue ($P = 0.13$), whereas 7/8 cases with +14 had gained the maternally inherited homologue ($P = 0.07$). However, subsequent analyses of additional cases, reported by Wilson et al.[49] and in article IV, showed no such skewness. Thus, a total of 24 cases with trisomy 14 have been investigated; 14 of these had maternal origin of the duplicated chromosome ($P = 0.54$). Furthermore, of the eight analyzed cases with +8, six had paternal origin of the gained chromosome ($P = 0.29$). Hence, we can exclude that

imprinting effects related to the parental origin of the duplicated chromosomes are of importance in hyperdiploid childhood ALL.

UPDs may arise as a consequence of the mechanism behind the formation of hyperdiploidy, but they may also occur independently, with a possible pathogenetic impact. Because of the resulting allelic imbalance for imprinted loci, formation of UPDs could be the pathogenetic equivalent to duplicating a chromosome. Support for the presence of UPDs in hyperdiploid cases came from an LOH study of chromosome 6 in ALL; UPD for this chromosome was quite frequent and was found in at least one hyperdiploid case.[204] To test if UPD is a common feature of hyperdiploid childhood ALL, all disomies for chromosomes X, 4, 6, 10, 14, 17, and 18 present in 27 cases, were analyzed in article IV. Only one of 29 such disomic chromosomes was a UPD, excluding the UPDs in a case that had originated via a near-haploid pathway. Furthermore, Irving et al.[191] investigated all chromosomes in three cases and detected UPD for chromosomes 4 and 6 in one of these. However, this case also displayed UPD for chromosomes 12 and 20, and may have arisen via a tetraploid state. Hence, UPDs for the commonly trisomic chromosomes, not associated with a mechanism for hyperdiploidy that results in UPDs, are rare in hyperdiploid ALL.

*Pathogenetic impact – gene dosage effects?*
The first study of global gene expression in hyperdiploid ALL was performed by Yeoh et al.[205], using a microarray platform containing 12,600 transcripts. Sixty-four of 327 investigated ALLs were hyperdiploid; these displayed a specific gene expression profile. Although Yeoh et al.[205] did not report a general overexpression of genes on the gained chromosomes, 70% of the class-defining genes were localized to chromosomes 21 and X, which are frequently gained in hyperdiploid cases. Interestingly, the chromosome X genes were overexpressed also in cases without this trisomy, indicating upregulation by alternative mechanisms.[205] In a further investigation of partly the same cases, Ross et al.[206], using a microarray platform containing 39,000 transcripts, showed that hyperdiploid childhood ALL was characterized by overexpressed genes, upregulated by a factor of two. This was presumed to result from the gained chromosomes.[206] In contrast, Qui et al.[207] detected no overexpression of genes at the tri-/tetrasomic chromosomes in nine hyperdiploid cases, but a specific gene expression pattern was noted. Gruszka-Westwood et al.[29] used comparative expressed sequence hybridization to detect regions of upregulation in hyperdiploid childhood ALL. They found that the gene

expression was correlated with copy number, with upregulation of genes at trisomic chromosomes and with genes at tetrasomies displaying higher expression than those at trisomies. Notably, not all regions in the gained chromosomes showed this pattern; instead, peaks of overexpression were seen. Furthermore, loci at disomic chromosomes were also de-regulated.[207]

None of the above-mentioned studies addressed the biologic significance of the discriminating genes in the hyperdiploid subgroup, and data about this are therefore scarce. In a recent investigation, van Delft et al.[208], analyzing 13 hyperdiploid childhood ALLs by gene expression microarrays, reported a common expression profile for such cases, those harboring the t(12;21)(p13;q22), and those with loss or gain of 12p or 21q, respectively. This may indicate that these cytogenetic sub-groups affect the same pathways.[208] Also, Kager et al.[209] detected a specific expression pattern for genes involved in the folate pathway in hyperdiploid childhood ALL.

In conclusion, hyperdiploid cases display a unique gene expression signature compared to other cytogenetic subgroups of child-hood ALL, reflecting, at least in part, upregulation of genes localized at the gained chromosomes. Little is known about the resulting effects on the biochemical pathways of the cell, and further studies are definitely warranted. However, the pathogenetic impact of hyperdiploidy in child-hood ALL may well be associated with dosage effects resulting from the tri- and tetrasomies.

*Conclusions and future directions*

During the last decades, numerous investigations have addressed hyper-diploidy in pediatric ALL. Today, the clinical features have been de-scribed and the genetic patterns are being elucidated. We know that hyperdiploidy frequently – possibly always – arises before birth and that it usually does so by a simultaneous gain of chromosomes in a single ab-normal mitosis. However, every new fact we learn about this cytogenetic subgroup seems to lead to more questions. What are the etiologic factors inducing hyperdiploidy already in utero? What are the cooperating genetic events needed for leukemogenesis? Do cases originating by different mechanisms have different biologic/clinical outcomes? Why are these specific chromosomes gained, especially if there is no gradual selection of chromosomes providing a proliferative advantage? Hopefully, future studies will address these questions and other enigmas concerning the hyperdiploid cytogenetic subtype in childhood ALL.

# SVENSK SAMMANFATTNING
# (SUMMARY IN SWEDISH)

*Hematologiska maligniteter* är samlingsnamnet för ett stort antal cancer-sjukdomar som involverar blodet och den blodbildande benmärgen. De inkluderar bl a de *kroniska* och *akuta leukemierna*, som tillsammans årligen drabbar ca 1000 personer i Sverige. Kroniska leukemier före-kommer huvudsakligen bland äldre personer, medan akut leukemi har en topp bland barn – där det är den vanligaste cancersjukdomen – och sedan uppvisar ökande incidens med åldern. Leukemier delas in i *myeloiska* och *lymfatiska*, beroende på vilka blodceller de involverar.

I princip alla celler i kroppen har 23 par *kromosomer*, som inne-håller vår arvsmassa – *generna*. Idag vet man att cancer beror på fel i cellens genetiska kontrollprogram. Detta innebär inte att all cancer är ärftlig – det är t ex mycket ovanligt med familjär förekomst av leukemi. Istället är det *förvärvade genetiska förändringar* som styr omvandlingen från en normal cell till en cancercell. Dessa avvikelser är inte medfödda, utan uppstår i en cell under individens levnad. Om en genetisk förändring innebär att cellen får en tillväxtfördel gentemot omkringliggande vävnad, så kommer dess dotterceller i "nästa generation" att bli proportionerligt sett fler. Normalt är det immunförsvarets uppgift att ta hand om okontrollerad tillväxt, men detta kan förhindras genom att ytterligare för-ändringar uppstår, som osynliggör de genetiskt skadade cellerna. Så småningom har de tillförskansat sig så många genetiska avvikelser att de utvecklas till cancerceller. I akuta leukemier innebär detta i praktiken att ett stort antal omogna blodceller helt eller delvis tar över benmärgen och blodet, med infektionskänslighet, blödningar och trötthet som följd.

Genetiska förändringar som syns på kromosomnivå hittas i mer än hälften av alla akuta leukemier. Identifieringen av dem är kliniskt mycket viktig, eftersom olika avvikelser ger information om vilken sjukdomstyp det handlar om samt påverkar prognosen och valet av behandlingsform. De genetiska förändringarna inkluderar bland annat omflyttningar av kromosomdelar – *translokationer* – som påverkar cellens kontrollgener. Sådana avvikelser är relativt välundersökta och forskningen kring dem har till och med lett till nya läkemedel. Dock är det lika vanligt att leukemicellerna har fått tillskott av kromosomer, s k *trisomier*. I motsats till translokationerna så vet man väldigt lite om hur trisomierna uppstår, hur de påverkar cellen och vad kopplingen till

leukemiutveckling är. Syftet med den här avhandlingen var att försöka finna svaren på de frågorna.

I de första två studierna undersöktes fall av akut myeloisk leukemi (AML) och myelodysplastiska syndrom (MDS) – en när-besläktad sjukdom som ibland övergår i AML – med trisomi för kromo-som 8 (*trisomi 8* eller *+8*) som enda kromosomavvikelse. Detta är en av de vanligaste genetiska förändringarna i AML och MDS, men trots det har man ingen bra förklaring till hur den påverkar cellen. En teori är att +8 i sig själv inte är tillräcklig för leukemiuppkomsten, utan att andra av-vikelser är nödvändiga. Eftersom trisomi 8 ofta ses som den enda för-ändringen på kromosomnivå, så skulle dessa eventuella extra genfel involvera så små bitar av kromosomerna att de inte syns med vanliga undersökningsmetoder. I studie I analyserades därför 13 fall av +8-positiv AML/MDS med fluorescent in situ hybridisering (FISH); en teknik där man använder sig av fluorescerande s k prober för att färga in olika kromosomdelar, som sedan kan studeras i speciella mikroskop. Med FISH kan man t ex se om små bitar av kromosomerna har bytt plats eller försvunnit. Med denna metod upptäcktes dock inte några ytterligare genetiska förändringar i de 13 fallen.

I studie II användes en annan teknik: ”array-baserad CGH”, som möjliggör upptäckt av tillskott eller förlust av små kromosombitar. Totalt studerades tio AML- och MDS-fall med synbarligen enbart trisomi 8. Undersökningen resulterade i identifiering av tolv små genetiska för-ändringar som inte hade upptäckts med sedvanlig kromosomanalys. Dessa fanns i totalt fem AML/MDS-fall, d v s i hälften av de undersökta fallen, och var troligtvis av betydelse för leukemiuppkomsten. Upp-täckten visar att det kan finnas ytterligare genetiska skador, som inte syns med vanlig kromosomanalys, i leukemiceller och stöder uppfattningen att enbart trisomi 8 inte är tillräcklig för leukemiutveckling.

I studie III och IV undersöktes s k *hyperdiploid* akut lymfatisk leukemi (ALL) hos barn. Hyperdiploidi, som innebär att cellerna inne-håller många extra kromosomer, d v s många trisomier, i ett specifikt mönster, är den vanligaste leukemitypen i den här åldersgruppen och är förknippad med en god prognos. Resultaten från en tidigare studie antydde att de extra kromosomerna troligtvis tillkom under en enda fel-aktig celldelning, men andra uppkomstmekanismer kunde inte säkert ute-slutas. Vi undersökte detta med molekylärgenetiska metoder i tio fall i studie III, och kunde bekräfta att en enda felaktig celldelning var det troligaste alternativet. För att helt kunna utesluta övriga mekanismer studerades i studie IV ytterligare 27 fall. Utifrån våra resultat kunde vi

dra slutsatsen att tidigare nämnda mekanism förekommer i den stora majoriteten av hyperdiploid barn-ALL, men att de extra kromosomerna kan tillkomma på andra sätt i en del av fallen.

       Vi studerade också den biologiska bakgrunden till att tillskott av kromosomer kan leda till leukemi. I varje kromosompar har de två kromosomerna ärvts från varsin förälder och vissa gener är olika aktiva beroende på om de nedärvts från modern eller fadern. En möjlighet var därför att det hade betydelse från vilken förälder de extra kromosomerna ursprungligen kom i hyperdiploid ALL. För att undersöka detta samlade vi in blodprov från föräldrar till barn med denna sjukdom i studie III och IV och gjorde med hjälp av dessa föräldraskapstest på trisomierna. Resultaten visade att teorin inte stämde: tillkomst av kromosomer sker oberoende av från vilken förälder de ursprungligen nedärvts. Samman-fattningsvis så har undersökningarna i den här avhandlingen lett till en större förståelse av sambanden mellan trisomier och leukemi och av hur sådana kromosomavvikelser uppstår i cancerceller.

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# REFERENCES

1.  PALLISTER C. *Blood. Physiology and pathophysiology*. 1994. Butterworth-Heinemann Ltd, Oxford.
2.  MITELMAN F, JOHANSSON B, and MERTENS F (Eds.). Mitelman Database of Chromosome Aberrations in Cancer. 2005. http://cgap.nci.nih.gov/ Chromosomes/Mitelman
3.  ROWLEY JD. Chromosome translocations: dangerous liaisons revisited. *Nat Rev Cancer* 2001;1:245-250.
4.  MITELMAN F, JOHANSSON B, and MERTENS F. Fusion genes and rearranged genes as a linear function of chromosome aberrations in cancer. *Nat Genet* 2004;36:331-334.
5.  HARRISON CJ. The management of patients with leukaemia: the role of cytogenetics in this molecular era. *Br J Haematol* 2000;108:19-30.
6.  GRIMWADE D and LO COCO F. Acute promyelocytic leukemia: a model for the role of molecular diagnosis and residual disease monitoring in directing treatment approach in acute myeloid leukemia. *Leukemia* 2002;16:1959-1973.
7.  DEININGER M, BUCHDUNGER E, and DRUKER BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood* 2005;105:2640-2653.
8.  JOHANSSON B, MERTENS F, and MITELMAN F. Primary vs. secondary neoplasia-associated chromosomal abnormalities – balanced rearrangements vs. genomic imbalances? *Genes Chromosomes Cancer* 1996;16:155-163.
9.  RABBITTS TH and STOCKS MR. Chromosomal translocation products engender new intracellular therapeutic technologies. *Nat Med* 2003;9:383-386.
10. HECHT JL and ASTER JC. Molecular biology of Burkitt's lymphoma. *J Clin Oncol* 2000;18:3707-3721.
11. KUROKAWA M and HIRAI H. Role of AML1/Runx1 in the pathogenesis of hematological malignancies. *Cancer Sci* 2003;94:841-846.
12. RAMSEY H, ZHANG D-E, RICHKIND K, BURCOGLU-O'RAL A, and HROMAS R. Fusion of AML1/Runx1 to copine VIII, a novel member of the copine family, in an aggressive acute myelogenous leukemia with t(12;21) translocation. *Leukemia* 2003;17:1665-1666.
13. RAMSEY H, CHRISTOPHERSON K, and HROMAS R. Forced expression of AML1-AMP19, a fusion transcript generated from a radiation-associated t(19;21) leukemia, blocks myeloid differentiation. *Leuk Res* 2004;28:863-868.
14. GRAUX C, COOLS J, MELOTTE C, QUENTMEIER H, FERRANDO A, LEVINE R, VERMEESCH JR, STUL M, DUTTA B, BOECKX N, BOSLY A, HEIMANN P, UYTTEBROECK A, MENTENS N, SOMERS R, MACLEOD RAF, DREXLER HG, LOOK AT, GILLILAND DG, MICHAUX L, VANDENBERGHE P, WLODARSKA I, MARYNEN P, and HAGEMEIJER A. Fusion of *NUP214* to *ABL1* on amplified episomes in T-cell acute lymphoblastic leukemia. *Nat Genet* 2004;36:1084-1089.
15. KOURLAS PJ, STROUT MP, BECKNELL B, VERONESE ML, CROCE CM, THEIL KS, KRAHE R, RUUTU T, KNUUTILA S, BLOOMFIELD CD, and CALIGIURI MA. Identification of a gene at 11q23 encoding a guanine nucleotide exchange

factor: evidence for its fusion with *MLL* in acute myeloid leukemia. *Proc Natl Acad Sci USA* 2000;97:2145-2150.

16. FU J-F, HSU J-J, TANG T-C, and SHIH L-Y. Identification of *CBL*, a proto-onco-gene at 11q23.3, as a novel *MLL* fusion partner in a patient with de novo acute myeloid leukemia. *Genes Chromosomes Cancer* 2003;37:214-219.

17. GOTLIB J, COOLS J, MALONE III JM, SCHRIER SL, GILLILAND DG, and COUTRÉ SE. The FIP1L1-PDGFRα fusion tyrosine kinase in hypereosinophilic syn-drome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. *Blood* 2004;103:2879-2891.

18. HEIM S. Is cancer cytogenetics reducible to the molecular genetics of cancer cells? *Genes Chromosomes Cancer* 1992;5:188-196.

19. STIREWALT DL and RADICH JP. The role of FLT3 in haematopoietic malignancies. *Nat Rev Cancer* 2003;3:650-665.

20. OSATO M. Point mutations in the *RUNX1/AML1* gene: another actor in RUNX leukemia. *Oncogene* 2004;23:4284-4296.

21. RAGHAVAN M, LILLINGTON DM, SKOULAKIS S, DEBERNARDI S, CHAPLIN T, FOOT NJ, LISTER TA, and YOUNG BD. Genome-wide single nucleotide poly-morphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. *Cancer Res* 2005;65:375-378.

22. GORLETTA TA, GASPARINI P, D'ELIOS MM, TRUBIA M, PELICCI PG, and DI FIORE PP. Frequent loss of heterozygosity without loss of genetic material in acute myeloid leukemia with a normal karyotype. *Genes Chromosomes Cancer* 2005; In press.

23. CLAUS R and LÜBBERT M. Epigenetic targets in hematopoietic malignancies. *Oncogene* 2003;22:6489-6496.

24. CALDAS C and BRENTON JD. Sizing up miRNAs as cancer genes. *Nat Med* 2005;11:712-714.

25. CALIN GA, DUMITRU CD, SHIMIZU M, BICHI R, ZUPO S, NOCH E, ALDLER H, RATTAN S, KEATING M, RAI K, RASSENTI L, KIPPS T, NEGRINI M, BULLRICH F, and CROCE CM. Frequent deletions and down-regulation of micro-RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002;99:15524-15529.

26. FORESTIER E, JOHANSSON B, BORGSTRÖM G, KERNDRUP G, JOHANNSSON J, and HEIM S. Cytogenetic findings in a population-based series of 787 child-hood acute lymphoblastic leukemias from the Nordic countries. *Eur J Haematol* 2000;64:194-200.

27. FITZPATRICK DR. Transcriptional consequences of autosomal trisomy: primary gene dosage with complex downstream effects. *Trends Genet* 2005;21:249-253.

28. SCHOCH C, KOHLMANN A, DUGAS M, KERN W, HIDDEMANN W, SCHNITTGER S, and HAFERLACH T. Genomic gains and losses influence expression levels of genes located within the affected regions: a study on acute myeloid leukemias with trisomy 8, 11, or 13, monosomy 7, or deletion 5q. *Leukemia* 2005;19:1224-1228.

29. GRUSZKA-WESTWOOD AM, HORSLEY SW, MARTINEZ-RAMIREZ A, HARRISON CJ, KEMPSKI H, MOORMAN AV, ROSS FM, GRIFFITHS M, GREAVES MF, and KEARNEY L. Comparative expressed sequence hybridization studies of high-

hyperdiploid childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 2004;41:191-202.

30. UPENDER MB, HABERMANN JK, MCSHANE LM, KORN EL, BARRETT JC, DIFILIPPANTONIO MJ, and RIED T. Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. *Cancer Res* 2004;64:6941-6949.

31. MAO R, ZIELKE CL, ZIELKE HR, and PEVSNER J. Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. *Genomics* 2003;81:457-467.

32. FITZPATRICK DR, RAMSAY J, MCGILL NI, SHADE M, CAROTHERS AD, and HASTIE ND. Transcriptome analysis of human autosomal trisomy. *Hum Mol Genet* 2002;11:3249-3256.

33. GOLUB TR, BARKER GF, BOHLANDER SK, HIEBERT SW, WARD DC, BRAY-WARD P, MORGAN E, RAIMONDI SC, ROWLEY JD, and GILLILAND DG. Fusion of the *TEL* gene on 12p13 to the *AML1* gene on 21q22 in acute lymphoblastic leukemia. *Proc Natl Acad Sci USA* 1995;92:4917-4921.

34. GURBUXANI S, VYAS P, and CRISPINO JD. Recent insights into the mechanisms of myeloid leukemogenesis in Down syndrome. *Blood* 2004;103:399-406.

35. WECHSLER J, GREENE M, MCDEVITT MA, ANASTASI J, KARP JE, LE BEAU MM, and CRISPINO JD. Acquired mutations in *GATA1* in the megakaryoblastic leukemia of Down syndrome. *Nat Genet* 2002;32:148-152.

36. MUNDSCHAU G, GURBUXANI S, GAMIS AS, GREENE ME, ARCECI RJ, and CRISPINO JD. Mutagenesis of *GATA1* is an initiating event in Down syndrome leukemogenesis. *Blood* 2003;101:4298-4300.

37. BIANCHI AB, ALDAZ CM, and CONTI CJ. Nonrandom duplication of the chromosome bearing a mutated Ha-*ras-1* allele in mouse skin tumors. *Proc Natl Acad Sci USA* 1990;87:6902-6906.

38. CALIGIURI MA, STROUT MP, SCHICHMAN SA, MRÓZEK K, ARTHUR DC, HERZIG GP, BAER MR, SCHIFFER CA, HEINONEN K, KNUUTILA S, NOUSIAINEN T, RUUTU T, BLOCK AW, SCHULMAN P, PEDERSEN-BJERGAARD J, CROCE CM, and BLOOMFIELD CD. Partial tandem duplication of *ALL1* as a recurrent molecular defect in acute myeloid leukemia with trisomy 11. *Cancer Res* 1996;56:1418-1425.

39. CALIGIURI MA, STROUT MP, OBERKIRCHER AR, YU F, DE LA CHAPELLE A, and BLOOMFIELD CD. The partial tandem duplication of *ALL1* in acute myeloid leukemia with normal cytogenetics or trisomy 11 is restricted to one chromosome. *Proc Natl Acad Sci USA* 1997;94:3899-3902.

40. ZHUANG Z, PARK W-S, PACK S, SCHMIDT L, VORTMEYER AO, PAK E, PHAM T, WEIL RJ, CANDIDUS S, LUBENSKY IA, LINEHAN WM, ZBAR B, and WEIRICH G. Trisomy 7-harbouring non-random duplication of the mutant *MET* allele in hereditary papillary renal carcinomas. *Nat Genet* 1998;20:66-69.

41. BEGHINI A, RIPAMONTI CB, CASTORINA P, PEZZETTI L, DONEDA L, CAIROLI R, MORRA E, and LARIZZA L. Trisomy 4 leading to duplication of a mutated *KIT* allele in acute myeloid leukemia with mast cell involvement. *Cancer Genet Cytogenet* 2000;119:26-31.

42. BEGHINI A, RIPAMONTI CB, CAIROLI R, CAZZANIGA G, COLAPIETRO P, ELICE F, NADALI G, GRILLO G, HAAS OA, BIONDI A, MORRA E, and LARIZZA L. KIT activating mutations: incidence in adult and pediatric acute myeloid leukemia,

and identification of an internal tandem duplication. *Haematologica* 2004;89:920-925.

43.  HUANG SC, KOCH CA, VORTMEYER AO, PACK SD, LICHTENAUER UD, MANNAN P, LUBENSKY IA, CHROUSOS GP, GAGEL RF, PACAK K, and ZHUANG Z. Duplication of the mutant *RET* allele in trisomy 10 or loss of the wild-type allele in multiple endocrine neoplasia type 2-associated pheochromocytomas. *Cancer Res* 2000;60:6223-6226.

44.  KAWAKAMI T, OKAMOTO K, SUGIHARA H, HATTORI T, REEVE AE, OGAWA O, and OKADA Y. The MET proto-oncogene is not a major target for the gain of chromosome 7 in testicular germ-cell tumors of adolescents. *Virchows Arch* 2004;444:480-481.

45.  POWELL H, CURTIS A, BOWN N, and TAYLOR P. No correlation between trisomy 13 and *FLT3* duplication in acute myeloid leukemia. *Cancer Genet Cytogenet* 2005;156:92-93.

46.  JOHANSSON B, MOORMAN AV, and SECKER-WALKER LM. Derivative chromosomes of 11q23-translocations in hematologic malignancies. *Leukemia* 1998;12:828-833.

47.  JIRTLE RL. Genomic imprinting and cancer. *Exp Cell Res* 1999;248:18-24.

48.  HAAS OA. Is genomic imprinting involved in the pathogenesis of hyperdiploid and haploid acute lymphoblastic leukemia of childhood? *Acta Genet Med Gemellol (Roma)* 1996;45:239-242.

49.  WILSON JL and MORISON IM. No evidence for preferential maternal origin of duplicated chromosome 14 in hyperdiploid ALL. *Blood* 2005;105:1837.

50.  SCHROEDER WT, CHAO L-Y, DAO DD, STRONG LC, PATHAK S, RICCARDI V, LEWIS WH, and SAUNDERS GF. Nonrandom loss of maternal chromosome 11 alleles in Wilms tumors. *Am J Hum Genet* 1987;40:413-420.

51.  SANSON M, LEURAUD P, MARIE Y, DELATTRE J-Y, and HOANG-XUAN K. Preferential loss of paternal 19q, but not 1p, alleles in oligodendrogliomas. *Ann Neurol* 2002;52:105-107.

52.  RAJAGOPALAN H and LENGAUER C. Aneuploidy and cancer. *Nature* 2004;432:338-341.

53.  GIEHL M, FABARIUS A, FRANK O, HOCHHAUS A, HAFNER M, HEHLMANN R, and SEIFARTH W. Centrosome aberrations in chronic myeloid leukemia correlate with stage of disease and chromosomal instability. *Leukemia* 2005;19:1192-1197.

54.  NEBEN K, GIESECKE C, SCHWEIZER S, HO AD, and KRÄMER A. Centrosome aberrations in acute myeloid leukemia are correlated with cytogenetic risk profile. *Blood* 2003;101:289-291.

55.  HANKS S, COLEMAN K, REID S, PLAJA A, FIRTH H, FITZPATRICK D, KIDD A, MÉHES K, NASH R, ROBIN N, SHANNON N, TOLMIE J, SWANSBURY J, IRRTHUM A, DOUGLAS J, and RAHMAN N. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in *BUB1B*. *Nat Genet* 2004;36:1159-1161.

56.  JACQUEMONT S, BOCÉNO M, RIVAL JM, MÉCHINAUD F, and DAVID A. High risk of malignancy in mosaic variegated aneuploidy syndrome. *Am J Med Genet* 2002;109:17-21.

57.  PLAJA A, VENDRELL T, SMEETS D, SARRET E, GILI T, CATALÀ V, MEDIANO C, and SCHERES JMJC. Variegated aneuploidy related to premature centromere

division (PCD) is expressed in vivo and is a cancer-prone disease. *Am J Med Genet* 2001;98:216-223.

58.     OHSHIMA K, HARAOKA S, YOSHIOKA S, HAMASAKI M, FUJIKI T, SUZUMIYA J, KAWASAKI C, KANDA M, and KIKUCHI M. Mutation analysis of mitotic checkpoint genes (hBUB1 and hBUBR1) and microsatellite instability in adult T-cell leukemia/lymphoma. *Cancer Lett* 2000;158:141-150.

59.     HOFMANN W-K, MILLER CW, TSUKASAKI K, TAVOR S, IKEZOE T, HOELZER D, TAKEUCHI S, and KOEFFLER HP. Mutation analysis of the DNA-damage checkpoint gene CHK2 in myelodysplastic syndromes and acute myeloid leukemias. *Leuk Res* 2001;25:333-338.

60.     HANGAISHI A, OGAWA S, QIAO Y, WANG L, HOSOYA N, YUJI K, IMAI Y, TAKEUCHI K, MIYAWAKI S, and HIRAI H. Mutations of Chk2 in primary hematopoietic neoplasms. *Blood* 2002;99:3075-3077.

61.     TSUKASAKI K, MILLER CW, GREENSPUN E, ESHAGHIAN S, KAWABATA H, FUJIMOTO T, TOMONAGA M, SAWYERS C, SAID JW, and KOEFFLER HP. Mutations in the mitotic check point gene, MAD1L1, in human cancers. *Oncogene* 2001;20:3301-3305.

62.     TRASK BJ. Fluorescence *in situ* hybridization: applications in cytogenetics and gene mapping. *Trends Genet* 1991;7:149-154.

63.     TANKE HJ, WIEGANT J, VAN GIJLSWIJK RPM, BEZROOKOVE V, PATTENIER H, HEETEBRIJ RJ, TALMAN EG, RAAP AK, and VROLIJK J. New strategy for multi-colour fluorescence *in situ* hybridisation: COBRA: COmbined Binary RAtio labelling. *Eur J Hum Genet* 1999;7:2-11.

64.     TCHINDA J, VOLPERT S, MCNEIL N, NEUMANN T, KENNERKNECHT I, RIED T, BÜCHNER T, SERVE H, BERDEL WE, HORST J, and HILGENFELD E. Multicolor karyotyping in acute myeloid leukemia. *Leuk Lymphoma* 2003;44:1843-1853.

65.     PINKEL D and ALBERTSON DG. Array comparative genomic hybridization and its applications in cancer. *Nat Genet* 2005;37:S11-17.

66.     ADINOLFI M, PERTL B, and SHERLOCK J. Rapid detection of aneuploidies by microsatellite and the quantitative fluorescent polymerase chain reaction. *Prenat Diagn* 1997;17:1299-1311.

67.     HULTÉN MA, DHANJAL S, and PERTL B. Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF-PCR. *Reproduction* 2003;126:279-297.

68.     MÜLLER S, O'BRIEN PCM, FERGUSON-SMITH MA, and WIENBERG J. Cross-species colour segmenting: a novel tool in human karyotype analysis. *Cytometry* 1998;33:445-452.

69.     CHUDOBA I, PLESCH A, LÖRCH T, LEMKE J, CLAUSSEN U, and SENGER G. High resolution multicolor-banding: a new technique for refined FISH analysis of human chromosomes. *Cytogenet Cell Genet* 1999;84:156-160.

70.     ELLIOTT MA, LETENDRE L, HANSON CA, TEFFERI A, and DEWALD GW. The prognostic significance of trisomy 8 in patients with acute myeloid leukemia. *Leuk Lymphoma* 2002;43:583-586.

71.     WOLMAN SR, GUNDACKER H, APPELBAUM FR, and SLOVAK ML. Impact of trisomy 8 (+8) on clinical presentation, treatment response, and survival in acute myeloid leukemia: a Southwest Oncology Group study. *Blood* 2002;100:29-35.

72.	PAULSSON K, SÄLL T, FIORETOS T, MITELMAN F, and JOHANSSON B. The incidence of trisomy 8 as a sole chromosomal aberration in myeloid malignancies varies in relation to gender, age, prior iatrogenic genotoxic exposure, and morphology. *Cancer Genet Cytogenet* 2001;130:160-165.

73.	SOLÉ F, ESPINET B, SANZ GF, CERVERA J, CALASANZ MJ, LUÑO E, PRIETO F, GRANADA I, HERNÁNDEZ JM, CIGUDOSA JC, DIEZ JL, BUREO E, MARQUÉS ML, ARRANZ E, RÍOS R, CLIMENT JAM, VALLESPÍ T, FLORENSA L, and WOESSNER S. Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. *Br J Haematol* 2000;108:346-356.

74.	GREENBERG P, COX C, LEBEAU MM, FENAUX P, MOREL P, SANZ G, SANZ M, VALLESPI T, HAMBLIN T, OSCIER D, OHYASHIKI K, TOYAMA K, AUL C, MUFTI G, and BENNETT J. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood* 1997;89:2079-2088.

75.	BERNASCONI P, KLERSY C, BONI M, CAVIGLIANO PM, CALATRONI S, GIARDINI I, ROCCA B, ZAPPATORE R, CARESANA M, QUARNA J, LAZZARINO M, and BERNASCONI C. Incidence and prognostic significance of karyotype abnormalities in *de novo* primary myelodysplastic syndromes: a study on 331 patients from a single institution. *Leukemia* 2005;19:1424-1431.

76.	SCHIFFER CA, LEE EJ, TOMIYASU T, WIERNIK PH, and TESTA JR. Prognostic impact of cytogenetic abnormalities in patients with de novo acute nonlymphocytic leukemia. *Blood* 1989;73:263-270.

77.	HAFERLACH T, SCHOCH C, SCHNITTGER S, KERN W, LÖFFLER H, and HIDDEMANN W. Distinct genetic patterns can be identified in acute monoblastic and acute monocytic leukaemia (FAB AML M5a and M5b): a study of 124 patients. *Br J Haematol* 2002;118:426-431.

78.	SECOND MIC COOPERATIVE STUDY GROUP. Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukaemias. *Br J Haematol* 1988;68:487-494.

79.	HRŬSÁK O and PORWIT-MACDONALD A. Antigen expression patterns reflecting genotype of acute leukemias. *Leukemia* 2002;16:1233-1258.

80.	CASASNOVAS RO, CAMPOS L, MUGNERET F, CHARRIN C, BÉNÉ MC, GARAND R, FAVRE M, SARTIAUX C, CHAUMAREL I, BERNIER M, FAURE G, and SOLARY E. Immunophenotypic patterns and cytogenetic anomalies in acute non-lymphoblastic leukemia subtypes: a prospective study of 432 patients. *Leukemia* 1998;12:34-43.

81.	MAURITZSON N, ALBIN M, RYLANDER L, BILLSTRÖM R, AHLGREN T, MIKOCZY Z, BJÖRK J, STRÖMBERG U, NILSSON PG, MITELMAN F, HAGMAR L, and JOHANSSON B. Pooled analysis of clinical and cytogenetic features in treatment-related and *de novo* adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976-1993 and on 5098 unselected cases reported in the literature 1974-2001. *Leukemia* 2002;16:2366-2378.

82.	ALBIN M, BJÖRK J, WELINDER H, TINNERBERG H, MAURITZSON N, JOHANSSON B, BILLSTRÖM R, STRÖMBERG U, MIKOCZY Z, AHLGREN T, NILSSON P-G, MITELMAN F, and HAGMAR L. Acute myeloid leukemia and clonal chromosome aberrations in relation to past exposure to organic solvents. *Scand J Work Environ Health* 2000;26:482-491.

83.    ZHANG L, LAN Q, GUO W, LI G, YANG W, HUBBARD AE, VERMEULEN R, RAPPAPORT SM, YIN S, ROTHMAN N, and SMITH MT. Use of OctoChrome fluorescence in situ hybridization to detect specific aneuploidy among all 24 chromosomes in benzene-exposed workers. *Chem Biol Interact* 2005;153-154:117-122.

84.    DAVICO L, SACERDOTE C, CICCONE G, PEGORARO L, KERIM S, PONZIO G, and VINEIS P. Chromosome 8, occupational exposures, smoking, and acute nonlymphocytic leukemias: a population-based study. *Cancer Epidemiol Biomarkers Prev* 1998;7:1123-1125.

85.    BJÖRK J, ALBIN M, MAURITZSON N, STRÖMBERG U, JOHANSSON B, and HAGMAR L. Smoking and acute myeloid leukemia: associations with morphology and karyotypic patterns and evaluation of dose-response relations. *Leuk Res* 2001;25:865-872.

86.    WOLSTENHOLME J. Confined placental mosaicism for trisomies 2, 3, 7, 8, 9, 16, and 22: their incidence, likely origins, and mechanisms for cell lineage compartmentalization. *Prenat Diagn* 1996;16:511-524.

87.    KARADIMA G, BUGGE M, NICOLAIDIS P, VASSILOPOULOS D, AVRAMOPOULOS D, GRIGORIADOU M, ALBRECHT B, PASSARGE E, ANNERÉN G, BLENNOW E, CLAUSEN N, GALLA-VOUMVOURAKI A, TSEZOU A, KITSIOU-TZELI S, HAHNEMANN JM, HERTZ JM, HOUGE G, KUKLIK M, MACEK M, LACOMBE D, MILLER K, MONCLA A, PAJARES IL, PATSALIS PC, PRIEUR M, VEKEMANS M, VON BEUST G, BRONDUM-NIELSEN K, and PETERSEN MB. Origin of nondisjunction in trisomy 8 and trisomy 8 mosaicism. *Eur J Hum Genet* 1998;6:432-438.

88.    JAMES RS and JACOBS PA. Molecular studies of the aetiology of trisomy 8 in spontaneous abortions and the liveborn population. *Hum Genet* 1996;97:283-286.

89.    BUYSE ML. *Birth defects encyclopedia*. 1990. Blackwell scientific publications, Oxford.

90.    SEGHEZZI L, MASERATI E, MINELLI A, DELLAVECCHIA C, ADDIS P, LOCATELLI F, ANGIONI A, BALLONI P, MIANO C, CAVALLI P, DANESINO C, and PASQUALI F. Constitutional trisomy 8 as first mutation in multistep carcinogenesis: clinical, cytogenetic, and molecular data on three cases. *Genes Chromosomes Cancer* 1996;17:94-101.

91.    SATGE D and VAN DEN BERGHE H. Aspects of the neoplasms observed in patients with constitutional autosomal trisomy. *Cancer Genet Cytogenet* 1996;87:63-70.

92.    WELBORN J. Constitutional chromosome aberrations as pathogenetic events in hematologic malignancies. *Cancer Genet Cytogenet* 2004;149:137-153.

93.    HAAS OA and SEYGER M. Hypothesis: meiotic origin of trisomic neoplasms. *Cancer Genet Cytogenet* 1993;70:112-116.

94.    SECKER-WALKER LM and FITCHETT M. Constitutional and acquired trisomy 8. *Leuk Res* 1995;19:737-740.

95.    ZOLLINO M, GENUARDI M, BAJER J, TORNESELLO A, MASTRANGELO S, ZAMPINO G, MASTRANGELO R, and NERI G. Constitutional trisomy 8 and myelodysplasia: report of a case and review of the literature. *Leuk Res* 1995;19:733-736.

96.     MASERATI E, APRILI F, VINANTE F, LOCATELLI F, AMENDOLA G, ZATTERALE A, MILONE G, MINELLI A, BERNARDI F, LO CURTO F, and PASQUALI F. Trisomy 8 in myelodysplasia and acute leukemia is constitutional in 15-20% of cases. *Genes Chromosomes Cancer* 2002;33:93-97.

97.     SCHOCH C, HAASE D, FONATSCH C, HAFERLACH T, LÖFFLER H, SCHLEGELBERGER B, HOSSFELD D-K, BECHER R, SAUERLAND MC, HEINECKE A, WÖRMANN B, BÜCHNER T, and HIDDEMANN W. The significance of trisomy 8 in *de novo* acute myeloid leukaemia: the accompanying chromosome aberrations determine the prognosis. *Br J Haematol* 1997;99:605-611.

98.     GRIMWADE D, WALKER H, OLIVER F, WHEATLEY K, HARRISON C, HARRISON G, REES J, HANN I, STEVENS R, BURNETT A, and GOLDSTONE A. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. *Blood* 1998;92:2322-2333.

99.     BYRD JC, LAWRENCE D, ARTHUR DC, PETTENATI MJ, TANTRAVAHI R, QUMSIYEH M, STAMBERG J, DAVEY FR, SCHIFFER CA, and BLOOMFIELD CD. Patients with isolated trisomy 8 in acute myeloid leukemia are not cured with cytarabine-based chemotherapy: results from Cancer and Leukemia Group B 8461. *Clin Cancer Res* 1998;4:1235-1241.

100.   BYRD JC, MROZEK K, DODGE RK, CARROLL AJ, EDWARDS CG, ARTHUR DC, PETTENATI MJ, PATIL SR, RAO KW, WATSON MS, KODURU PRK, MOORE JO, STONE RM, MAYER RJ, FELDMAN EJ, DAVEY FR, SCHIFFER CA, LARSON RA, and BLOOMFIELD CD. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood* 2002;100:4325-4336.

101.   FARAG SS, ARCHER KJ, MROZEK K, VARDIMAN JW, CARROLL AJ, PETTENATI MJ, MOORE JO, KOLITZ JE, MAYER RJ, STONE RM, LARSON RA, and BLOOMFIELD CD. Isolated trisomy of chromosomes 8, 11, 13 and 21 is an adverse prognostic factor in adults with *de novo* acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. *Int J Oncol* 2002;21:1041-1051.

102.   HUNTLY BJP and GILLILAND DG. Leukaemia stem cells and the evolution of cancer-stem-cell research. *Nat Rev Cancer* 2005;5:311-321.

103.   MEHROTRA B, GEORGE TI, KAVANAU K, AVET-LOISEAU H, MOORE II D, WILLMAN CL, SLOVAK ML, ATWATER S, HEAD DR, and PALLAVICINI MG. Cytogenetically aberrant cells in the stem cell compartment (CD34$^+$lin$^-$) in acute myeloid leukemia. *Blood* 1995;86:1139-1147.

104.   PARLIER V, TIAINEN M, BERIS P, MIESCHER PA, KNUUTILA S, and JOTTERAND BELLOMO M. Trisomy 8 detection in granulomonocytic, erythrocytic and megakaryocytic lineages by chromosomal *in situ* suppression hybridization in a case of refractory anaemia with ringed sideroblasts complicating the course of paroxysmal nocturnal haemoglobinuria. *Br J Haematol* 1992;81:296-304.

105.   ANASTASI J, FENG J, LE BEAU MM, LARSON RA, ROWLEY JD, and VARDIMAN JW. Cytogenetic clonality in myelodysplastic syndromes studied with fluorescence in situ hybridization: lineage, response to growth factor therapy, and clone expansion. *Blood* 1993;81:1580-1585.

106.   BILLSTRÖM R, JOHANSSON B, STRÖMBECK B, EL-RIFAI W, LARRAMENDY M, OLOFSSON T, MITELMAN F, and KNUUTILA S. Clonal CD5-positive B lympho-

cytes in myelodysplastic syndrome with systemic vasculitis and trisomy 8. *Ann Hematol* 1997;74:37-40.

107.    ABRUZZESE E, BUSS D, RAINER R, PETTENATI MJ, and RAO PN. Progression of a myelodysplastic syndrome to pre-B acute lymphoblastic leukemia: a case report and cell lineage study. *Ann Hematol* 1996;73:35-38.

108.    SAITOH K, MIURA I, TAKAHASHI N, and MIURA AB. Fluorescence in situ hybridization of progenitor cells obtained by fluorescence-activated cell sorting for the detection of cells affected by chromosome abnormality trisomy 8 in patients with myelodysplastic syndromes. *Blood* 1998;92:2886-2892.

109.    NILSSON L, ÅSTRAND-GRUNDSTRÖM I, ANDERSON K, ARVIDSSON I, HOKLAND P, BRYDER D, KJELDSEN L, JOHANSSON B, HELLSTRÖM-LINDBERG E, HAST R, and JACOBSEN SEW. Involvement and functional impairment of the CD34$^+$CD38$^-$Thy-1$^+$ hematopoietic stem cell pool in myelodysplatic syndromes with trisomy 8. *Blood* 2002;100:259-267.

110.    JOHANSSON B, MERTENS F, and MITELMAN F. Secondary chromosomal abnormalities in acute leukemias. *Leukemia* 1994;8:953-962.

111.    JENNINGS BA and MILLS KI. c-*myc* locus amplification and the acquisition of trisomy 8 in the evolution of chronic myeloid leukaemia. *Leuk Res* 1998;22:899-903.

112.    OUDAT R, KHAN Z, and GLASSMAN AB. Detection of trisomy 8 in philadelphia chromosome-positive CML patients using conventional cytogenetic and inter-phase fluorescence in situ hybridization techniques and its relation to c-*myc* involvement. *Ann Clin Lab Sci* 2001;31:68-74.

113.    MERTENS F, JOHANSSON B, and MITELMAN F. The pathogenetic significance of acquired trisomy 8 is not reducible to amplification of a single chromosome band. *Cancer Genet Cytogenet* 1995;83:176-177.

114.    VIRTANEVA K, WRIGHT FA, TANNER SM, YUAN B, LEMON WJ, CALIGIURI MA, BLOOMFIELD CD, DE LA CHAPELLE A, and KRAHE R. Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. *Proc Natl Acad Sci USA* 2001;98:1124-1129.

115.    PAULSSON K, LASSEN C, KURIC N, BILLSTRÖM R, FIORETOS T, TANKE HJ, and JOHANSSON B. *MYC* is not overexpressed in a case of chronic myelomonocytic leukemia with *MYC*-containing double minutes. *Leukemia* 2003;17:813-815.

116.    STORLAZZI CT, FIORETOS T, PAULSSON K, STRÖMBECK B, LASSEN C, AHLGREN T, JULIUSSON G, MITELMAN F, ROCCHI M, and JOHANSSON B. Identification of a commonly amplified 4.3 Mb region with overexpression of *C8FW*, but not *MYC* in *MYC*-containing double minutes in myeloid malignancies. *Hum Mol Genet* 2004;13:1479-1485.

117.    VEY N, MOZZICONACCI M-J, GROULET-MARTINEC A, DEBONO S, FINETTI P, CARBUCCIA N, BEILLARD E, DEVILARD E, ARNOULET C, COSO D, SAINTY D, XERRI L, STOPPA A-M, LAFAGE-POCHITALOFF M, NGUYEN C, HOULGATTE R, BLAISE D, MARANINCHI D, BIRG F, BIRNBAUM D, and BERTUCCI F. Identification of new classes among acute myelogenous leukaemias with normal karyotype using gene expression profiling. *Oncogene* 2004;23:9381-9391.

118.    BULLINGER L, DÖHNER K, BAIR E, FRÖHLING S, SCHLENK RF, TIBSHIRANI R, DÖHNER H, and POLLACK JR. Use of gene-expression profiling to identify

prognostic subclasses in adult acute myeloid leukemia. *N Engl J Med* 2004;350:1605-1616.

119.   CHEN G, ZENG W, MIYAZATO A, BILLINGS E, MACIEJEWSKI JP, KAJIGAYA S, SLOAND EM, and YOUNG NS. Distinctive gene expression profiles of CD34 cells from patients with myelodysplastic syndrome characterized by specific chromosomal abnormalities. *Blood* 2004;104:4210-4218.

120.   MINELLI A, MASERATI E, GIUDICI G, TOSI S, OLIVIERI C, BONVINI L, DE FILIPPI P, BIONDI A, LO CURTO F, PASQUALI F, and DANESINO C. Familial partial monosomy 7 and myelodysplasia: different parental origin of the monosomy 7 suggests action of a mutator gene. *Cancer Genet Cytogenet* 2001;124:147-151.

121.   MINELLI A, MASERATI E, ROSSI G, BERNARDO ME, DE STEFANO P, CECCHINI MP, VALLI R, ALBANO V, PIERANI P, LESZL A, SAINATI L, LO CURTO F, DANESINO C, LOCATELLI F, and PASQUALI F. Familial platelet disorder with propensity to acute myelogenous leukemia: genetic heterogeneity and progression to leukemia via acquisition of clonal chromosome anomalies. *Genes Chromosomes Cancer* 2004;40:165-171.

122.   MASERATI E, MINELLI A, MENNA G, CECCHINI MP, BERNARDO ME, ROSSI G, DE FILIPPI P, LO CURTO F, DANESINO C, LOCATELLI F, and PASQUALI F. Familial myelodysplastic syndromes, monosomy 7/trisomy 8, and mutator effects. *Cancer Genet Cytogenet* 2004;148:155-158.

123.   MORISON IM, PATON CJ, and CLEVERLEY SD. The imprinted gene and parent-of-origin effect database. *Nucleic Acids Res* 2001;29:275-276.

124.   KARANJAWALA ZE, KÄÄRIÄINEN H, GHOSH S, TANNENBAUM J, MARTIN C, ALLY D, TUOMILEHTO J, VALLE T, and COLLINS FS. Complete maternal isodisomy of chromosome 8 in an individual with an early-onset ileal carcinoid tumor. *Am J Med Genet* 2000;93:207-210.

125.   BENLIAN P, FOUBERT L, GAGNÉ E, BERNARD L, DE GENNES JL, LANGLOIS S, ROBINSON W, and HAYDEN M. Complete paternal isodisomy for chromosome 8 unmasked by lipoprotein lipase deficiency. *Am J Hum Genet* 1996;59:431-436.

126.   JOHANSSON B, BILLSTRÖM R, BROBERG K, FIORETOS T, NILSSON P-G, AHLGREN T, MALM C, SAMUELSSON BO, and MITELMAN F. Cytogenetic polyclonality in hematologic malignancies. *Genes Chromosomes Cancer* 1999;24:222-229.

127.   MOHR B, BORNHÄUSER M, THIEDE C, SCHÄKEL U, SCHAICH M, ILLMER T, PASCHEBERG U, and EHNINGER G. Comparison of spectral karyotyping and conventional cytogenetics in 39 patients with acute myeloid leukemia and myelodysplastic syndrome. *Leukemia* 2000;14:1031-1038.

128.   HILGENFELD E, PADILLA-NASH H, McNEIL N, KNUTSEN T, MONTAGNA C, TCHINDA J, HORST J, LUDWIG W-D, SERVE H, BÜCHNER T, BERDEL WE, SCHRÖCK E, and RIED T. Spectral karyotyping and fluorescence *in situ* hybridization detect novel chromosomal aberrations, a recurring involvement of chromosome 21 and amplification of the *MYC* oncogene in acute myeloid leukaemia M2. *Br J Haematol* 2001;113:305-317.

129.   KERNDRUP GB and KJELDSEN E. Acute leukemia cytogenetics: an evaluation of combining G-band karyotyping with multi-color spectral karyotyping. *Cancer Genet Cytogenet* 2001;124:7-11.

130. BROWN J, SARACOGLU K, UHRIG S, SPEICHER MR, EILS R, and KEARNEY L. Subtelomeric chromosome rearrangements are detected using an innovative 12-color FISH assay (M-TEL). *Nat Med* 2001;7:497-501.

131. HELLER A, BRECEVIC L, GLASER M, LONCAREVIC IF, GEBHART E, CLAUSSEN U, and LIEHR T. Trisomy 8 as the sole chromosomal aberration in myelocytic malignancies: a comprehensive molecular cytogenetic analysis reveals no cryptic aberrations. *Cancer Genet Cytogenet* 2003;146:81-83.

132. DIAZ MO, LE BEAU MM, HARDEN A, and ROWLEY JD. Trisomy 8 in human hematologic neoplasia and the c-*myc* and c-*mos* oncogenes. *Leuk Res* 1985;9:1437-1442.

133. LANGABEER SE, GRIMWADE D, WALKER H, ROGERS JR, BURNETT AK, GOLDSTONE AH, and LINCH DC. A study to determine whether trisomy 8, deleted 9q and trisomy 22 are markers of cryptic rearrangements of *PML/RARα*, *AML1/ETO* and *CBFB/MYH11* respectively in acute myeloid leukaemia. *Br J Haematol* 1998;101:338-340.

134. LEROY H, ROUMIER C, HUYGHE P, BIGGIO V, FENAUX P, and PREUDHOMME C. *CEBPA* point mutations in hematological malignancies. *Leukemia* 2005;19:329-334.

135. ANDERSSON A, JOHANSSON B, LASSEN C, MITELMAN F, BILLSTRÖM R, and FIORETOS T. Clinical impact of internal tandem duplications and activating point mutations in *FLT3* in acute myeloid leukemia in elderly patients. *Eur J Haematol* 2004;72:307-313.

136. BOWEN DT, FREW ME, HILLS R, GALE RE, WHEATLEY K, GROVES MJ, LANGABEER SE, KOTTARIDIS PD, MOORMAN AV, BURNETT AK, and LINCH DC. *RAS* mutation in acute myeloid leukemia is associated with distinct cyto-genetic subgroups but does not influence outcome in patients < 60 yrs. *Blood* 2005: in press.

137. HARADA H, HARADA Y, NIIMI H, KYO T, KIMURA A, and INABA T. High incidence of somatic mutations in the *AML1/RUNX1* gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. *Blood* 2004;103:2316-2324.

138. MOORMAN AV, CLARK R, FARRELL DM, HAWKINS JM, MARTINEAU M, and SECKER-WALKER LM. Probes for hidden hyperdiploidy in acute lymphoblastic leukaemia. *Genes Chromosomes Cancer* 1996;16:40-45.

139. MOORMAN AV, RICHARDS SM, MARTINEAU M, CHEUNG KL, ROBINSON HM, JALALI GR, BROADFIELD ZJ, HARRIS RL, TAYLOR KE, GIBSON BES, HANN IM, HILL FGH, KINSEY SE, EDEN TOB, MITCHELL CD, and HARRISON CJ. Outcome heterogeneity in childhood high-hyperdiploid acute lymphoblastic leukemia. *Blood* 2003;102:2756-2762.

140. RAIMONDI SC, PUI C-H, HANCOCK ML, BEHM FG, FILATOV L, and RIVERA GK. Heterogeneity of hyperdiploid (51-67) childhood acute lymphoblastic leukemia. *Leukemia* 1996;10:213-224.

141. HEEREMA NA, SATHER HN, SENSEL MG, ZHANG T, HUTCHINSON RJ, NACHMAN JB, LANGE BJ, STEINHERZ PG, BOSTROM BC, REAMAN GH, GAYNON PS, and UCKUN FM. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (> 50 chromosomes). *J Clin Oncol* 2000;18:1876-1887.

142.   ELGHEZAL H, LE GUYADER G, RADFORD-WEISS I, PEROT C, VAN DEN AKKER J, EYDOUX P, VEKEMANS M, and ROMANA SP. Reassessment of childhood B-lineage lymphoblastic leukemia karyotypes using spectral analysis. *Genes Chromosomes Cancer* 2001;30:383-392.

143.   NORDGREN A, FARNEBO F, JOHANSSON B, HOLMGREN G, FORESTIER E, LARSSON C, SÖDERHÄLL S, NORDENSKJÖLD M, and BLENNOW E. Identification of numerical and structural chromosome aberrations in 15 high hyperdiploid childhood acute lymphoblastic leukemias using spectral karyotyping. *Eur J Haematol* 2001;66:297-304.

144.   NORDGREN A, HEYMAN M, SAHLÉN S, SCHOUMANS J, SÖDERHÄLL S, NORDENSKJÖLD M, and BLENNOW E. Spectral karyotyping and interphase FISH reveal abnormalities not detected by conventional G-banding. Implications for treatment stratification of childhood acute lymphoblastic leukaemia: detailed analysis of 70 cases. *Eur J Haematol* 2002;68:31-41.

145.   TEIXEIRA MR and HEIM S. Multiple numerical chromosome aberrations in cancer: what are their causes and what are their consequences? *Semin Cancer Biol* 2005;15:3-12.

146.   ANDREASSON P, HÖGLUND M, BÉKÁSSY AN, GARWICZ S, HELDRUP J, MITELMAN F, and JOHANSSON B. Cytogenetic and FISH studies of a single center consecutive series of 152 childhood acute lymphoblastic leukemias. *Eur J Haematol* 2000;65:40-51.

147.   CHESSELLS JM, SWANSBURY GJ, REEVES B, BAILEY CC, and RICHARDS SM. Cytogenetics and prognosis in childhood lymphoblastic leukaemia: results of MRC UKALL X. *Br J Haematol* 1997;99:93-100.

148.   GROUPE FRANÇAIS DE CYTOGÉNÉTIQUE HÉMATOLOGIQUE. Collaborative study of karyotypes in childhood acute lymphoblastic leukemias. *Leukemia* 1993;7:10-19.

149.   SECKER-WALKER LM, CHESSELLS JM, STEWART EL, SWANSBURY GJ, RICHARDS S, and LAWLER SD. Chromosomes and other prognostic factors in acute lymphoblastic leukaemia: a long-term follow-up. *Br J Haematol* 1989;72:336-342.

150.   HEIM S, BÉKÀSSY AN, GARWICZ S, HELDRUP J, KRISTOFFERSSON U, MANDAHL N, WIEBE T, and MITELMAN F. Bone marrow karyotypes in 94 children with acute leukemia. *Eur J Haematol* 1990;44:227-233.

151.   PUI C-H, WILLIAMS DL, ROBERSON PK, RAIMONDI SC, BEHM FG, LEWIS SH, RIVERA GK, KALWINSKY DK, ABROMOWITCH M, CRIST WM, and MURPHY SB. Correlation of karyotype and immunophenotype in childhood acute lymphoblastic leukemia. *J Clin Oncol* 1988;6:56-61.

152.   JOHANSSON B, MERTENS F, and MITELMAN F. Clinical and biological importance of cytogenetic abnormalities in childhood and adult acute lympho-blastic leukemia. *Ann Med* 2004;36:492-503.

153.   YAGI T, HIBI S, TABATA Y, KURIYAMA K, TERAMURA T, HASHIDA T, SHIMIZU Y, TAKIMOTO T, TODO S, SAWADA T, and IMASHUKU S. Detection of clonotypic IGH and TCR rearrangements in the neonatal blood spots of infants and children with B-cell precursor acute lymphoblastic leukemia. *Blood* 2000;96:264-268.

154.   TAUB JW, KONRAD MA, GE Y, NABER JM, SCOTT JS, MATHERLY LH, and RAVINDRANATH Y. High frequency of leukemic clones in newborn screening

blood samples of children with B-precursor acute lymphoblastic leukemia. *Blood* 2002;99:2992-2996.

155.    PANZER-GRÜMAYER ER, FASCHING K, PANZER S, HETTINGER K, SCHMITT K, STÖCKLER-IPSIROGLU S, and HAAS OA. Nondisjunction of chromosomes leading to hyperdiploid childhood B-cell precursor acute lymphoblastic leukemia is an early event during leukemogenesis. *Blood* 2002;100:347-349.

156.    MAIA AT, VAN DER VELDEN VHJ, HARRISON CJ, SZCZEPANSKI T, WILLIAMS MD, GRIFFITHS MJ, VAN DONGEN JJM, and GREAVES MF. Prenatal origin of hyperdiploid acute lymphoblastic leukemia in identical twins. *Leukemia* 2003;17:2202-2206.

157.    MAIA AT, TUSSIWAND R, CAZZANIGA G, REBULLA P, COLMAN S, BIONDI A, and GREAVES M. Identification of preleukemic precursors of hyperdiploid acute lymphoblastic leukemia in cord blood. *Genes Chromosomes Cancer* 2004;40:38-43.

158.    WIEMELS JL, ZHANG Y, CHANG J, ZHENG S, METAYER C, ZHANG L, SMITH MT, MA X, SELVIN S, BUFFLER PA, and WIENCKE JK. *RAS* mutation is associated with hyperdiploidy and parental characteristics in pediatric acute lymphoblastic leukemia. *Leukemia* 2005;19:415-419.

159.    HJALGRIM LL, ROSTGAARD K, HJALGRIM H, WESTERGAARD T, THOMASSEN H, FORESTIER E, GUSTAFSSON G, KRISTINSSON J, MELBYE M, and SCHMIEGELOW K. Birth weight and risk for childhood leukemia in Denmark, Sweden, Norway, and Iceland. *J Natl Cancer Inst* 2004;96:1549-1556.

160.    THOMPSON JR, GERALD PF, WILLOUGHBY MLN, and ARMSTRONG BK. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. *Lancet* 2001;358:1935-1940.

161.    WANG X, THOMAS P, XUE J, and FENECH M. Folate deficiency induces aneuploidy in human lymphocytes in vitro – evidence using cytokinesis-blocked cells and probes specific for chromosomes 17 and 21. *Mutat Res* 2004;551:167-180.

162.    WIEMELS JL, SMITH RN, TAYLOR GM, EDEN OB, ALEXANDER FE, GREAVES MF, and UNITED KINGDOM CHILDHOOD CANCER STUDY investigators. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. *Proc Natl Acad Sci USA* 2001;98:4004-4009.

163.    FORESTIER E, JOHANSSON B, GUSTAFSSON G, BORGSTRÖM G, KERNDRUP G, JOHANNSSON J, and HEIM S. Prognostic impact of karyotypic findings in child-hood acute lymphoblastic leukaemia: a Nordic series comparing two treatment periods. *Br J Haematol* 2000;110:147-153.

164.    PUI C-H, RAIMONDI SC, DODGE RK, RIVERA GK, FUCHS LAH, ABROMOWITCH M, LOOK AT, FURMAN WL, CRIST WM, and WILLIAMS DL. Prognostic importance of structural chromosomal abnormalities in children with hyperdiploid (>50 chromosomes) acute lymphoblastic leukemia. *Blood* 1989;73:1963-1967.

165.    LAMPERT F. Cellulärer DNS-gehalt und chromosomenzahl bei der akuten leukämie im kindesalter und ihre bedeutung für chemotherapie und prognose. *Klin Wochenschr* 1967;45:763-768.

166. BLOOMFIELD CD, GOLDMAN AI, ALIMENA G, BERGER R, BORGSTRÖM GH, BRANDT L, CATOVSKY D, DE LA CHAPELLE A, DEWALD GW, GARSON OM, GARWICZ S, GOLOMB HM, HOSSFELD DK, LAWLER SD, MITELMAN F, NILSSSON P, PIERRE RV, PRIGOGINA PPE, ROWLEY JD, SAKURAI M, SANDBERG AA, SECKER-WALKER LM, TRICOT G, VAN DEN BERGHE H, VAN ORSHOVEN A, VUOPIO P, and WHANG-PENG J. Chromosomal abnormalities identify high-risk and low-risk patients with acute lymphoblastic leukemia. *Blood* 1986;67:415-420.

167. TRUEWORTHY R, SHUSTER J, LOOK T, CRIST W, BOROWITZ M, CARROLL A, FRANKEL L, HARRIS M, WAGNER H, HAGGARD M, MOSIJCZUK A, PULLEN J, STREUBER P, and LAND V. Ploidy of lymphoblasts is the strongest predictor of treatment outcome in B-progenitor cell acute lymphoblastic leukemia of childhood: a Pediatric Oncology Group study. *J Clin Oncol* 1992;10:606-613.

168. THIRD INTERNATIONAL WORKSHOP ON CHROMOSOMES IN LEUKEMIA. Chromosomal abnormalities and their clinical significance in acute lymphoblastic leukemia. *Cancer Res* 1983;43:868-873.

169. SECKER-WALKER LM, LAWLER SD, and HARDISTY RM. Prognostic implications of chromosomal findings in acute lymphoblastic leukaemia at diagnosis. *Br Med J* 1978;2:1529-1530.

170. JACKSON JF, BOYETT J, PULLEN J, BROCK B, PATTERSON R, LAND V, BOROWITZ M, HEAD D, and CRIST W. Favorable prognosis associated with hyperdiploidy in children with acute lymphocytic leukemia correlates with extra chromosome 6. A Pediatric Oncology Group study. *Cancer* 1990;66:1183-1189.

171. HARRIS MB, SHUSTER JJ, CARROLL A, LOOK AT, BOROWITZ MJ, CRIST WM, NITSCHKE R, PULLEN J, STEUBER CP, and LAND VJ. Trisomy of leukemic cell chromosomes 4 and 10 identifies children with B-progenitor cell acute lymphoblastic leukemia with a very low risk of treatment failure: a Pediatric Oncology Group study. *Blood* 1992;79:3316-3324.

172. SUTCLIFFE MJ, SHUSTER JJ, SATHER HN, CAMITTA BM, PULLEN J, SCHULTZ KR, BOROWITZ MJ, GAYNON PS, CARROLL AJ, and HEEREMA NA. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. *Leukemia* 2005;19:734-740.

173. PUI C-H, RAIMONDI SC, and WILLIAMS DL. Isochromosome 17q in childhood acute lymphoblastic leukemia: an adverse cytogenetic feature in association with hyperdiploidy? *Leukemia* 1988;2:222-225.

174. SYNOLD TW, RELLING MV, BOYETT JM, RIVERA GK, SANDLUND JT, MAHMOUD H, CRIST WM, PUI C-H, and EVANS WE. Blast cell methotrexate-polyglutamate accumulation in vivo differs by lineage, ploidy, and methotrexate dose in acute lymphoblastic leukemia. *J Clin Invest* 1994;94:1996-2001.

175. KASPERS GJL, SMETS LA, PIETERS R, VAN ZANTWIJK CH, VAN WERING ER, and VEERMAN AJP. Favorable prognosis of hyperdiploid common acute lymphoblastic leukemia may be explained by sensitivity to antimetabolites and other drugs: results of an in vitro study. *Blood* 1995;85:751-756.

176. WHITEHEAD VM, VUCHICH MJ, COOLEY LD, LAUER SJ, MAHONEY DH, SHUSTER JJ, PAYMENT C, KOCH PA, AKABUTU JJ, BOWEN T, KAMEN BA, RAVINDRANATH Y, EMAMI A, LOOK AT, BEARDSLEY GP, PULLEN DJ, and CAMITTA B. Accumulation of methotrexate polyglutamates, ploidy and trisomies of both chromosomes 4 and 10 in lymphoblasts from children with B-progenitor cell acute lymphoblastic leukemia: a Pediatric Oncology Group Study. *Leuk Lymphoma* 1998;31:507-519.

177. ZHANG L, TAUB JW, WILLIAMSON M, WONG SC, HUKKU B, PULLEN J, RAVINDRANATH Y, and MATHERLY LH. Reduced folate carrier gene expression in childhood acute lymphoblastic leukemia: relationship to immunophenotype and ploidy. *Clin Cancer Res* 1998;4:2169-2177.

178. BELKOV VM, KRYNETSKI EY, SCHUETZ JD, YANISHEVSKI Y, MASSON E, MATHEW S, RAIMONDI S, PUI C-H, RELLING MV, and EVANS WE. Reduced folate carrier expression in acute lymphoblastic leukemia: a mechanism for ploidy but not lineage differences in methotrexate accumulation. *Blood* 1999;93:1643-1650.

179. CHENG Q, YANG W, RAIMONDI SC, PUI C-H, RELLING MV, and EVANS WE. Karyotypic abnormalities create discordance of germline genotype and cancer cell phenotypes. *Nat Genet* 2005;37:878-882.

180. ZHANG Y, LU J, VAN DEN BERGHE J, and LEE S-H. Increased incidence of spontaneous apoptosis in the bone marrow of hyperdiploid childhood acute lymphoblastic leukemia. *Exp Hematol* 2002;30:333-339.

181. KUMAGAI M-A, MANABE A, PUI C-H, BEHM FG, RAIMONDI SC, HANCOCK ML, MAHMOUD H, CRIST WM, and CAMPANA D. Stroma-supported culture of childhood B-lineage acute lymphoblastic leukemia cells predicts treatment out-come. *J Clin Invest* 1996;97:755-760.

182. ITO C, KUMAGAI M-A, MANABE A, COUSTAN-SMITH E, RAIMONDI SC, BEHM FG, MURTI KG, RUBNITZ JE, PUI C-H, and CAMPANA D. Hyperdiploid acute lymphoblastic leukemia with 51 to 65 chromosomes: a distinct biological entity with a marked propensity to undergo apoptosis. *Blood* 1999;93:315-320.

183. LARRAMENDY M, EL-RIFAI W, SAARINEN U, ALITALO R, LUOMAHAARA S, and KNUUTILA S. Myeloid lineage involvement in acute lymphoblastic leukemia: a morphology antibody chromosomes (MAC) study. *Exp Hematol* 1995;23:1563-1567.

184. KASPRZYK A, HARRISON CJ, and SECKER-WALKER LM. Investigation of clonal involvement of myeloid cells in Philadelphia-positive and high hyperdiploid acute lymphoblastic leukemia. *Leukemia* 1999;13:2000-2006.

185. QUIJANO CA, MOORE II D, ARTHUR D, FEUSNER J, WINTER SS, and PALLAVICINI MG. Cytogenetically aberrant cells are present in the $CD34^+CD33^-38^-19^-$ marrow compartment in children with acute lymphoblastic leukemia. *Leukemia* 1997;11:1508-1515.

186. GREAVES M. In utero origins of childhood leukaemia. *Early Hum Dev* 2005;81:123-129.

187. GALE KB, FORD AM, REPP R, BORKHARDT A, KELLER C, EDEN OB, and GREAVES MF. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *Proc Natl Acad Sci USA* 1997;94:13950-13954.

188.    SZCZEPANSKI T, WILLEMSE MJ, VAN WERING ER, VAN WEERDEN JF, KAMPS WA, and VAN DONGEN JJM. Precursor-B-ALL with $D_H$-$J_H$ gene rearrangements have an immature immunogenotype with a high frequency of oligoclonality and hyperdiploidy of chromosome 14. *Leukemia* 2001;15:1415-1423.

189.    ONODERA N, MCCABE NR, and RUBIN CM. Formation of a hyperdiploid karyotype in childhood acute lymphoblastic leukemia. *Blood* 1992;80:203-208.

190.    HAAS OA, ZEITLHOFER P, STREHL S, PFEILSTOECKER M, KOENIG M, and WEINHAEUSEL A. A novel DNA/RNA FISH X inactivation assay reveals a nonrandom, ploidy-dependent acquisition of the active and inactive X chromosomes in childhood hyperdiploid acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL). *ASH Annual Meeting Abstracts* 2004;104:1080.

191.    IRVING JAE, BLOODWORTH L, BOWN NP, CASE MC, HOGARTH LA, and HALL AG. Loss of heterozygosity in childhood acute lymphoblastic leukemia detected by genome-wide microarray single nucleotide polymorphism analysis. *Cancer Res* 2005;65:3053-3058.

192.    HARRISON CJ, MOORMAN AV, BROADFIELD ZJ, CHEUNG KL, HARRIS RL, JALALI GR, ROBINSON HM, BARBER KE, RICHARDS SM, MITCHELL CD, EDEN TOB, HANN IM, HILL FGH, KINSEY SE, GIBSON BES, LILLEYMAN J, VORA A, GOLDSTONE AH, FRANKLIN IM, DURRANT J, and MARTINEAU M. Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. *Br J Haematol* 2004;125:552-559.

193.    OSHIMURA M, FREEMAN AI, and SANDBERG AA. Chromosomes and causation of human cancer and leukemia. XXIII. Near-haploidy in acute leukemia. *Cancer* 1977;40:1143-1148.

194.    GIBBONS B, MACCALLUM P, WATTS E, ROHATINER AZS, WEBB D, KATZ FE, SECKER-WALKER LM, TEMPERLEY IJ, HARRISON CJ, CAMPBELL RH, NASH R, BROADBENT V, and CHESSELLS JM. Near haploid acute lymphoblastic leukemia: seven new cases and a review of the literature. *Leukemia* 1991;5:738-743.

195.    STAMBERG J, SHENDE A, and LANZKOWSKY P. Somatic shift to homozygosity for a chromosomal polymorphism in a child with acute lymphoblastic leukemia. *Blood* 1986;67:350-353.

196.    ONODERA N, MCCABE NR, NACHMAN JB, JOHNSON FL, LE BEAU MM, ROWLEY JD, and RUBIN CM. Hyperdiploidy arising from near-haploidy in childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 1992;4:331-336.

197.    MA SK, CHAN GCF, WAN TSK, LAM CK, HA SY, LAU YL, and CHAN LC. Near-haploid common acute lymphoblastic leukaemia of childhood with a second hyperdiploid line: a DNA ploidy and fluorescence *in-situ* hybridization study. *Br J Haematol* 1998;103:750-755.

198.    STARK B, JEISON M, GOBUZOV R, KRUG H, GLASER-GABAY L, LURIA D, EL-HASID R, HARUSH MB, AVRAHAMI G, FISHER S, STEIN J, ZAIZOV R, and YANIV I. Near haploid childhood acute lymphoblastic leukemia masked by hyperdiploid line: detection by fluorescence in situ hybridization. *Cancer Genet Cytogenet* 2001;128:108-113.

199.    KANEKO Y and KNUDSON AG. Mechanism and relevance of ploidy in neuroblastoma. *Genes Chromosomes Cancer* 2000;29:89-95.

200.    TAKETANI T, TAKI T, SUGITA K, FURUICHI Y, ISHII E, HANADA R, TSUCHIDA M, SUGITA K, IDA K, and HAYASHI Y. *FLT3* mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with *MLL* rearrangements and pediatric ALL with hyperdiploidy. *Blood* 2004;103:1085-1088.

201.    ARMSTRONG SA, MABON ME, SILVERMAN LB, LI A, GRIBBEN JG, FOX EA, SALLAN SE, and KORSMEYER SJ. FLT3 mutations in childhood acute lymphoblastic leukemia. *Blood* 2004;103:3544-3546.

202.    TARTAGLIA M, MARTINELLI S, CAZZANIGA G, CORDEDDU V, IAVARONE I, SPINELLI M, PALMI C, CARTA C, PESSION A, ARICÒ M, MASERA G, BASSO G, SORCINI M, GELB BD, and BIONDI A. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic *PTPN11* mutations to leukemogenesis in childhood acute leukemia. *Blood* 2004;104:307-313.

203.    ZHENG S, MA X, ZHANG L, GUNN L, SMITH MT, WIEMELS JL, LEUNG K, BUFFLER PA, and WIENCKE JK. Hypermethylation of the 5' CpG island of the *FHIT* gene is associated with hyperdiploid and translocation-negative subtypes of pediatric leukemia. *Cancer Res* 2004;64:2000-2006.

204.    MCEVOY CRE, MORLEY AA, and FIRGAIRA FA. Evidence for whole chromosome 6 loss and duplication of the remaining chromosome in acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 2003;37:321-325.

205.    YEOH E-J, ROSS ME, SHURTLEFF SA, WILLIAMS WK, PATEL D, MAHFOUZ R, BEHM FG, RAIMONDI SC, RELLING MV, PATEL A, CHENG C, CAMPANA D, WILKINS D, ZHOU X, LI J, LIU H, PUI C-H, EVANS WE, NAEVE C, WONG L, and DOWNING JR. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell* 2002;1:133-143.

206.    ROSS ME, ZHOU X, SONG G, SHURTLEFF SA, GIRTMAN K, WILLIAMS WK, LIU H-C, MAHFOUZ R, RAIMONDI SC, LENNY N, PATEL A, and DOWNING JR. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. *Blood* 2003;102:2951-2959.

207.    QIU J, GUNARATNE P, PETERSON LE, KHURANA D, WALSHAM N, LOULSEGED H, KARNI RJ, ROUSSEL E, GIBBS RA, MARGOLIN JF, and GINGRAS MC. Novel potential ALL low-risk markers revealed by gene expression profiling with new high-throughput SSH-CCS-PCR. *Leukemia* 2003;17:1891-1900.

208.    VAN DELFT FW, BELLOTTI T, LUO Z, JONES LK, PATEL N, YIANNIKOURIS O, HILL AS, HUBANK M, KEMPSKI H, FLETCHER D, CHAPLIN T, FOOT N, YOUNG BD, HANN IM, GAMMERMAN A, and SAHA V. Prospective gene expression analysis accurately subtypes acute leukaemia in children and establishes a commonality between hyperdiploidy and t(12;21) in acute lymphoblastic leukaemia. *Br J Haematol* 2005;130:26-35.

209.    KAGER L, CHEOK M, YANG W, ZAZA G, CHENG Q, PANETTA JC, PUI C-H, DOWNING JR, RELLING MV, and EVANS WE. Folate pathway gene expression differs in subtypes of acute lymphoblastic leukemia and influences methotrexate pharmacodynamics. *J Clin Invest* 2005;115:110-117.