Cytogenetic Abnormalities

Chromosomal, FISH and Microarray-Based Clinical Reporting

Susan Mahler Zneimer

WILEY Blackwell

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Dedication

To my husband, Martin Chetlen, for his guidance, love and support, without which this book neither could nor would ever have been written.

And to my parents, Nadine and Joel, who raised me to think, and to imagine all the possibilities. May their memory be forever a blessing.

To all three, I raise my glass and say… thank you!

Contents

Preface

Numerous books and an untold number of publications in the scientific literature describe various chromosome abnormalities, both common and rare. One can search for any chromosome abnormality in a book index or an online scientific database and find every possible chromosome change that has been identified. Most of these abnormalities are reported, rightly so, in order to make a correlation with a clinical phenotype, diagnosis or prognosis of a disease. In other words, the emphasis has always been on directly correlating chromosome abnormalities with disease.

However, to date, little emphasis has been placed on standardizing how to designate chromosome abnormalities with their nomenclature and the related interpretation of these results with patient diagnoses. This book attempts to address the lack of standardization for writing the nomenclature of chromosome abnormalities and interpretive comments regarding those abnormalities. With over 250 cytogenetic laboratories in the United States alone, and maybe 500 laboratories worldwide, it is indeed time to develop more established guidelines for writing and interpreting cytogenetic, fluorescence *in situ* hybridization (FISH) and microarray test results.

The International System for Human Cytogenetic Nomenclature (ISCN) is the only resource (albeit a great one) to use in order to designate clear nomenclature in writing test results. Even so, if one is to compare a cytogenetic result from 10 different laboratories in the designation of a marker chromosome, for example, there could easily be five different ways to either write the ISCN nomenclature or interpret the result.

This book will be useful to cytogeneticists who write test results, to other geneticists, physicians, allied professionals and scientists who need to read these reports or use them in their clinical and/or research endeavors, and to those medical and human genetic students who are required to understand cytogenetic test results.

In this book, for each normal and abnormal cytogenetic result, the ISCN nomenclature and an interpretive comment with related recommendations are given. These abnormalities are divided among constitutional disorders and acquired malignancies, and are described by their genetic composition, genetic nomenclature and associated clinical features. Each chapter also contains a bibliography from which the information was obtained as well as pertinent research articles, databases and/or online web addresses for the reader's reference.

This book attempts to discuss as many genetic and malignant diseases as possible that have a known and prominent underlying genetic defect; however, it is not possible to list all the cytogenetic abnormalities in one manuscript. Readers who wish to see other abnormalities not included in the manuscript are encouraged to contact the author for reference in a future edition of the book.

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I have spent most of the day putting in a comma and the rest of the day taking it out.

Oscar Wilde

About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/zneimer/cytogenetic

The website includes:

- PDFs of all Example Report Boxes from the book for downloading. The Example Reports are the most important downloads that are needed for the book
- Powerpoints of all figures from the book for downloading
- PDFs of all tables from the book for downloading.

Introduction

Genetic testing is complex, and it is often difficult to understand the meaning of its results. One part of genetic testing is cytogenetic testing, often called chromosome analysis, which uses whole cells that are grown in culture in the laboratory to isolate DNA and identify differences of the chromosomes that would yield a genetic abnormality and lead to a genetic disorder or cancer. Cytogenetic analysis requires extensive manipulation of cells taken from an individual's body to isolate and analyze the chromosomes microscopically for the identification of chromosome aberrations. The complexity of this testing continues with writing a comprehensive and cohesive laboratory report that contains the correct information and correct nomenclature, and is understandable by the professional community that is conveying these results to patients. All too often, without assistance from genetic counselors, the professional receiving cytogenetic results from the genetics laboratory does not understand the nomenclature or the interpretive comments that explain a cytogenetics abnormality. These results are then not appropriately conveyed to the patient, widening the divide between medical science and the general population.

This book begins with an overview of genetics in general, cytogenetics in particular, and how its significance pertains to the general population. The discussion continues in subsequent chapters, in which specific cytogenetic abnormalities are discussed, and includes how cytogenetic reports are written for each abnormality, and how professionals and individuals with these disorders should interpret cytogenetic results.

Overview of cytogenetic testing in the laboratory

Cytogenetic testing is the study of chromosomes and their genetic composition, which is studied at different genetic levels. The "Gestalt" view of cytogenetics is the largest overview of chromosomes in which the banding level is important in identifying gross versus subtle, but visible, genetic changes. This level is generally referred to as standard or conventional cytogenetics. Conventional cytogenetics allows for the identification of large DNA changes that are visible on a chromosome, at least 1 million base pairs, whether the genetic change is a balanced or unbalanced abnormality. It is still the method of choice for many types of indications for genetic testing, such as cancer diagnosis and prognosis,

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history of spontaneous abortions, newborn dysmorphology, prenatal diagnosis and endocrinology disorders. There are limitations to conventional cytogenetics, one of which is the inability to visualize small abnormalities under a microscope, <1–5 million base pairs, eliminating the possibility of identifying submicroscopic genetic abnormalities. This limitation led to the development of new methodologies, including fluorescence *in situ* hybridization and microarray techniques, which enable the identification of smaller genetic changes and do not depend on the level of chromosome banding and morphology.

The next level of chromosome analysis is the level at which DNA probes can be used to identify regions of a chromosome using *in situ* hybridization (ISH). Although the first ISH analysis was done using radioactive tritium as a DNA probe, now the conventional approach uses fluorescent dyes attached to a small segment of DNA, hundreds to thousands of DNA base pairs long, that is specific to chromosomal regions of interest. This fluorescence *in situ* hybridization (FISH) is very useful in identifying DNA change on the chromosome that is specific to a disease region, a locus-specific region or a part of a chromosome that is used for chromosomal identification, such as a centromere, subtelomere or whole chromosome paint probe. FISH is very useful in identifying deletions, duplications and rearrangements of small disease regions where DNA probes can be made, or for chromosomal identification, when the presence of a chromosome is unidentifiable by standard chromosome analysis. FISH analysis also has its limitations, due to the small range of DNA size that supports a probe for hybridization. This range is generally from DNA segments from 1000 to 200,000 base pairs long. Another limitation of FISH is the need to know which region of a chromosome with which to probe. This is a targeted DNA test of the genome and only segments of interest are utilized in this methodology. Only with whole chromosome paint probes will the total genome be visible with the FISH methodology.

The next level of chromosome analysis is microarray technology. This method allows for a whole genome analysis at the molecular level in which small segments of DNA probes (oligo DNA probes) down to single base pair analyses (single nucleotide polymorphisms, SNPs) are used to identify all the segments of a genome for DNA imbalances in individuals. This methodology has become quite prevalent in all aspects of cytogenetic analysis, since large and small imbalances, including unbalanced rearrangements, duplications and deletions of any size, can be identified in an individual. Therefore, this type of analysis is replacing many aspects of the standard chromosome and FISH analyses, especially when specific indications for cytogenetic testing are known to be submicroscopic, and no clear disease state is in the differential diagnosis.

Table 1 Levels of DNA resolution from standard chromosome analysis by specimen type

Specimen type	Average band level	Average number of genes per band	Average number of base pairs per band	Band width in Mb
Bone marrow	350	100	9×10^6	9
Prenatal (AF and CVS)	450	75	7×10^6	7
Routine blood	550	60	6×10^6	6
High-resolution blood	800	45	4×10^6	4

AF, amniotic fluid; CVS, chorionic villus sampling.

Table 2 Abnormality detection by methodology

Abnormality	G-band	FISH (targeted sites)	Chromosome microarray analysis (CMA)
Whole chromosome imbalance (aneuploidy)	+	+	+
Balanced rearrangement (reciprocal translocations, inversions)	+	+	-
Deletions, duplications >10 Mb	+	+	+
Deletions, duplications <5~10 Mb (submicroscopic)	-	+	+
Mosaicism, if present at these levels	>20%	>5%	>20%
Marker (unidentifiable) chromosomes (non-mosaic)	+	+	+
Triploidy	+	+	+

FISH, fluorescence *in situ* hybridization.

The most significant use of microarray technology is for non-specific disorders, such as indications of autism spectrum disorders, mental impairment, developmental delay and brain or other organ dysfunction. It has also become prevalent for prenatal diagnosis and cancer disorders when many non-random, recurrent genetic changes are possible or, for example, in leukemias where many cytogenetic changes are known to be the underlying genetic change causing disease. A single microarray analysis is a good method to identify any of the possible abnormalities of gain or loss of genetic material known to be involved in specific diseases. This is in contrast to the many FISH probes that may be needed to test for a single disease or chromosome analysis, which is difficult to perform on neoplastic cells.

Microarray analysis, however, also has some limitations, including that it is most effective for identifying unbalanced rearrangements, whereas balanced rearrangements are not detectable (though this will probably be developed for clinical use in the near future). Microarray analysis is also too new to be the standard of care for most indications for genetic testing. However, ongoing development of this methodology will most likely make this type of testing more prevalent in the future.

See Tables 1 and 2 for a summary of the detection of chromosome abnormalities at each level and methodology employed.

Laboratory procedures for each type of methodology can be found in other sources, which are listed at the end of this chapter.

Genetic testing in most countries is generally governed by at least one agency. Some information regarding governmental and other regulatory agency requirements is provided but regulations vary depending on the state and country or residence of the laboratory or patient. Each government and agency has specific guidelines or laws that guide the laboratory for ethical, quality and monetary aspects. Other regulations in the United States include statements regarding whether a test is FDA approved or is an assay specific reagent (ASR) or for research use only (RUO). For the needed statement on reports, see Part 1, Section 2, where this is applicable.

Other rules and regulations will be discussed throughout the book when applicable.

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Part 1 Constitutional Analyses

Section 1 Chromosome Analysis

CHAPTER 1

Components of a standard cytogenetics report, normal results and culture failures

1.1 Components of a standard cytogenetics report

All cytogenetic reports should have specific information which helps to standardize that each laboratory is performing a minimum standard of competency and accuracy of results. Clinical laboratory improvement amendments (CLIA), College of American Pathologists (CAP) and various US states have placed requirements on each report. The information below is required for CLIA, CAP, NY State and CA State for regulatory compliance.

- Specimen type
- Indication for testing
- Number of cells counted
- Number of cells analyzed
- Number of cells karyotyped
- Banding technique
- ISCN nomenclature
- Interpretation

1.1.1 Specimen type

Specimen type refers to the source of tissue that is being analyzed for cytogenetic testing. The most common specimen types are:

- amniotic fluid and chorionic villus sampling (CVS) for prenatal studies
- peripheral blood for studies of liveborn individuals
- fetal tissue for products of conception (fetal demise) studies
- bone marrow, bone core or peripheral blood for leukemias
- bone marrow or lymph nodes for lymphomas
- muscle or skin biopsies for possible mosaic studies
- tumor biopsies for acquired or inherited malignancies.

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1.1.2 Indication for testing

Obtaining relevant clinical information about the patient is important in order to correlate cytogenetic results with the diagnosis. It sometimes becomes necessary for the laboratory to determine the appropriate set-up conditions of the specimen and the types of testing to perform, due to the various possibilities that exist. Therefore, in order for the laboratory to know what specific testing to perform, it needs all relevant patient information. Without the necessary patient and family clinical information, it may become a guessing game for the laboratory on the correct processing step to take. This is especially significant when it applies to cancer cytogenetics. Since certain cancer cells, including acute leukemias and myeloid disorders, divide continuously and do not require a B-cell or T-cell mitogen stimulant for cells to go through mitosis, the cultures that are initiated should be unstimulated 24-hour and 48-hour cultures. This is in contrast to chronic leukemias and other lymphoproliferative disorders, which do better with a B- or T-cell mitogen (e.g. IL4, TPA) to stimulate the cells to divide to have enough metaphases for analysis and which contain the abnormal cell type rather than normal lymphocytes. Also, knowing if acute lymphoblastic leukemia (ALL) is an indication for a patient will require only direct, overnight or 24-hour unstimulated cultures for analysis. Otherwise, there will be an overgrowth of normal cells dividing by the second day, and the abnormal lymphoblasts that are indicative of ALL will die off and not be present for analysis.

Culture initiation or set-up is also specific for the tumor type in question. No one culture medium is sufficient for all tumor types and so the culture medium should be specifically tailored for the proper growth of the abnormal tumor cells. For a guide on cancer cell culture media and growth factors for neoplastic cell growth, see the bibliography for detailed information.

1.1.3 Number of cells counted and analyzed

Counted cells refer to identifying a single cell and counting the number of chromosomes present plus identifying the sex chromosomes of that cell. Analyzed cells refer to identifying each chromosome homolog, band for band, to determine if any abnormalities exist within any of the chromosomes present.

Colonies refer to amniotic fluid cells that are cultured *in situ* on a small culture vessel, such as a coverslip. Colonies originate from single amniotic fluid cells that will grow and divide near each other in a colony, visibly separated from other originating amniotic fluid colonies. This type of culture allows for a greater distinction of progenitor cells in analysis versus allowing cells to congregate, grow and divide without spatial distinction, in which there is no knowledge of which cells are progenitor cells and which are the result of cell division and clones of progenitor cells. Without colonies, the cells in culture may be growing and dividing from only a very few hardy cells, and could possibly result in only a small number of original cells being analyzed, excluding possible mosaicism at a lower level.

The standard number of cells to be counted and analyzed depends on the specimen type. See Table 1.1 for a guide to the most common guidelines for cells counted and analyzed.

Table 1.1 Standard number of cells counted and analyzed per specimen type

	Postnatal peripheral blood	Prenatal amniotic fluid	Prenatal chorionic villus sampling	Neoplastic bone marrow and blood	Fetal demise and liveborn tissues	Neoplastic tumors	Mosaic studies
Cells counted	20	15 colonies or 20 cells	20	20	20	20–30	30–50
Cells analyzed	5	5 colonies or 5 cells	5	20	5	20–30	5

1.1.4 Number of cells karyotyped

The number of cells to be karyotyped is generally two per cell line. Exceptions to this rule include karyotyping only one cell of sideline clones in a neoplastic study, which will be discussed in greater detail in the cancer section of the book. More than two cells may be karyotyped if an abnormality is subtle and requires more than two cells to clarify the abnormality present.

1.1.5 Banding techniques

The standard banding techniques include those that clearly distinguish the significant bands identified by the International System for Human Cytogenetic Nomenclature (ISCN). The most common banding techniques which show the best banding patterns include G-banding, R-banding and Q-banding. Each technique uses different staining procedures to visualize the differential staining of cytosine/guanine (CG)-rich and adenosine/thymine (AT)-rich DNA. In each staining procedure, the bands observed are the same, but are visualized by AT with dark bands and CG with light bands or vice versa.

Other banding techniques are used to enhance specific regions of the chromosome, such as the centromere with C-banding, satellite regions of acrocentric chromosomes with nuclear organizer region (NOR) staining or telomeric regions with T-banding.

For a comprehensive discussion of banding techniques, refer to the bibliography at the end of the chapter.

1.1.6 Band levels

The banding level refers to an estimated total number of black, gray and white bands throughout the genome as it would appear in an ideogram of each chromosome. In the ISCN 2013 edition, on pages 16–31, ideograms of the chromosomes are described by band levels. There are a few reports in the literature of standardizing approaches to count the total number of bands in a karyotype. One approach is to count bands including the telomere, centromere and all the dark and light bands on chromosome 10. Table 1.2 details the correlation between the number of bands with the band level, using chromosome 10 as a reference.

Another approach for estimating band level is to count segments of specific chromosomes. For two different approaches, see a summary of these band estimations in Tables 1.3 and 1.4. Examples of cells with their corresponding karyotypes of each band level are depicted in Figure 1.1.

In a cytogenetics report, recording band level is generally a requirement. There is some debate on whether the highest band level observed in the best cell should be recorded in the report, or whether the band level of the best karyotype should be reported, or an average of the cells or karyotypes. Many laboratories record the best band level seen in a karyotype, which is easily documented for regulatory purposes and which may be corroborated if that karyotypic image is placed in the report itself.

Typically, the band level of a normal prenatal specimen of amniotic fluid and chorionic villus sampling is approximately 450 bands. For peripheral blood on liveborns, the typical band level is 500–550 bands. Hematological malignancies and solid tumors typically have fewer bands, generally in the range of 300–400 bands, reflecting the difficulty in analyzing dividing cells from abnormal cell types in malignancies.

When performing a high-resolution study, in which the minimum band level is 550–650 bands, a comment in the interpretation may be useful in order for the reader to know at what level chromosome analysis was achieved. It is also useful to report when the banding level did not reach the minimum requirement established by the laboratory or regulatory agency.

Table 1.2 Band level by counting the bands on chromosome 10 (adapted from Welborn and Welborn 1993)

Number of bands on chromosome 10	Estimated band level
12	375
13–14	400
15–16	425
17–18	450
19–21	475
22–23	500
24–25	525
26–28	550
29–30	600
31–32	650
33–34	700
35–36	750
37–38	800
39–40	850

Table 1.3a Tabulated band resolution of chromosomal segments (adapted from Josifek et al. 1991)

Chromosome region	Total bands counted for each band level		
	Band level 350–400	Band level 550	Band level 850
Chromosome region 1 from p31-p32	1	3	3
Whole chromosome 10	5	12	19
Short arm of chromosome 11	2	5	6
Long arm of chromosome 12	4–5	8	14
Whole chromosome X	6–8	12	18
Total bands counted	**18–21**	**40**	**60**

Table 1.3b Correlation of total bands with band level

Total bands	Band level
18	350
21	400
28	450
34	500
40	550
47	650
54	750
60	850