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HUMAN CYTOGENETICS: FROM CLASSICAL TO MOLECULAR KARYOTYPING

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Abstract — The establishment of human diploid chromosome number (Tjio and Levan, 1956), have led to the foundations of human cytogenetics. Since then, due to the rapid developments of new techniques for chromosome analysis, many other chromosome disorders were recognized. The introduction of banding techniques, yielding a highly reproducible banding pattern, allowed the reliable identification of every single chromosome and the precise diagnosis of structural and numerical chromosome aberrations. For more then 20 years, chromosome banding analysis has been the laboratory standard to identify structural and numerical chromosome abnormalities in pre- and postnatal cytogenetics as well as in cancer diagnosis. The resolution of classical cytogenetic techniques is limited to at best 4-5Mb and smaller chromosomal aberrations like small marker chromosomes, subtle translocations or complex chromosomal rearrangements often remain hidden. These diagnostic problems has been overcame to some degree, by the introduction of modern molecular cytogenetic techniques, like fluorescence in situ hybridization (FISH). The application of Comparative genomic hybridization (CGH) and microarray CGH has extended from screening for submicroscopic chromosomal imbalance in cancer cytogenetics to the detection of any type of gain or loss (resolution of 10-100 kb) of genetic material in dysmorphic and mentally retarded individuals with a normal conventional karyotype. To date around 20 000 chromosomal aberrations have been registered, and thay account for a large proportion of early spontaneous abortions, childhood disability and malignancy, as a consequence of somatic chromosome abnormalities. Current estimates show that 0.5-1.0% newborn infants will have a chromosome rearrangements recognizable either by conventional cytogenetics or by molecular cytogenetic method

Key words: Chromosomal disorders, cytogenetics, humans, syndromes

INTRODUCTION

The establishment of the human diploid chromosome number (Tjio and Levan, 1956) and definition of the chromosomal basis of Down syndrome (Lejeune, 1959) and other classical syndromes (DeGrouchy and Turleau, 1984) led to the foundation of human cytogenetics. Since then, due to the rapid development of new techniques for chromosome analysis, many other chromosome disorders have been recognized.

To date around 20,000 chromosomal aberrations have been registered, and they account for a large proportion of early spontaneous abortions, childhood disability, and malignancy resulting from somatic chromosome abnormalities (Mitelman, 1991; Jacobs et al., 1992; Schinzel, 1994).

Current estimates show that 0.5-1.0% of newborn infants will have a chromosome rearrangement recognizable either by conventional cytogenetics or by the molecular cytogenetic method (Turnpenny and Ellard, 2005).

CLASSICAL CYTOGENETICS IN DIAGNOSIS OF CHROMOSOME DISORDERS

In the past, progress in human cytogenetics depended largely on technological improvements that permitted a large number of good-quality metaphases to be obtained from cells cultured from different tissues. By 1959, several laboratories were engaged in the study of human chromosomes, and a common system of nomenclature was needed in order to improve communication between workers in the field of human cytogenetics. The first standard system of nomenclature of human mitotic chromosomes was proposed at the Denver Conference in 1960 (Denver Conference, 1960), and an improved standard system of nomenclature for description of the human chromosome complement and its abnormalities was adopted in Chicago at the Third International Congress on Human Genetics in 1966 (Chicago Conference, 1966). This system is now used throughout the world for description of non-banded human chromosomes.

Although identification of chromosomes by group was a significant achievement, most of extra structurally abnormal chromosomes could not be identified because chromosomes within a group resemble one another and only conventional methods of staining were available. The first advances in human chromosome identification came with THE introduction of banding techniques in chromosome analysis**.**

Banding techniques

The introduction of banding techniques, yielding a highly reproducible banding pattern, allowed the reliable identification of every single chromosome and precise diagnosis of structural and numerical chromosome aberrations.

In 1972, Caspersson and colleagues obtained the first banding picture of human chromosomes by staining with quinacrine mustard, which revealed Q-bands (Caspersson et al., 1972). Soon, several other techniques were developed that also showed chromosome bands: G-bands (Seabright, 1971; Drets and Shaw 1971) (Fig. 1), R-bands (Dutrilleaux and Lejeune, 1971; Bobrow and Madan, 1973) and C-bands (Arringhi and Hsu, 1971). Other new methods included nor-staining (Goodpasture and Bloom, 1975; Matsui and Sasaki, 1973) and sister chromatid differentiation (SCD) by 5-bromodeoxyuridin (BrDU) (Latt, 1975). Unequivocal identification of individual chromosomes and regions using various banding methods led to the realization that the existing system of nomenclature would no longer be adequate. The 1971 meeting in Paris resulted in the report of the Paris Conference

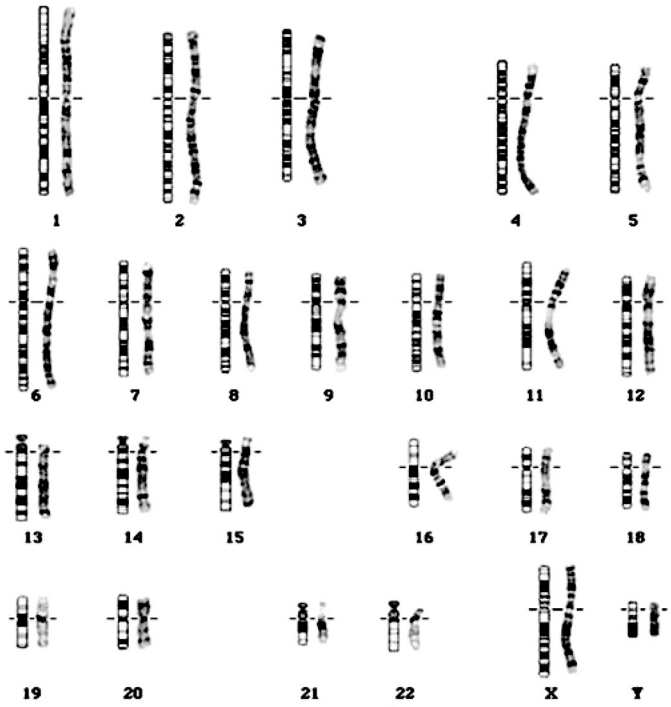

Fig. 1. G – banded karyotype of a male (Paris Conference, 1971).

(Paris Conference, 1971), which proposed an ideogram to depict the normal band morphology of each chromosome, as well as a unique numbering system to help describe different structural rearangements. A further change came about at the Fifth International Human Genetics Conference in Mexico City in 1976, when the International Standing Committee on Human Cytogenetic Nomenclature was elected. The Committee's work resulted in a document entitled: „An International System for Human Cytogenetic Nomenclature" (1978) or ISCN (1978), which included all decisions of the Denver, London, Chicago, and Paris Conferences, as well as a complete system of human cytogenetic nomenclature.

High-resolution banding techniques

The precision of chromosome banding analysis depends on the number of chromosome bands that can be distinguished (banding resolution).The chromosome banding resolution level, on the other hand, depends on the level of chromosome condensation: less condensed chromosomes contain a larger number of chromosome bands than more condensed chromosomes. The standard Paris ideogram contains about 315 bands in the haploid set of chromosomes. The very condensed chromosomes from bone marrow contain about 150-200 bands, and at this, or even lower, level of resolution the identification of chromosome aberrations may be uncertain. Thus, the analysis of less condensed chromosomes can increase the power of resolution (high-resolution banding). High-resolution chromosome banding can be obtained

by blocking cells in the S-phase, releasing the block, and then timing the subsequent harvest to obtain the maximum number of cells in prometaphase, when about 850 or more bands can be distinguished (Yunis, 1976; Pai and Thomas, 1980). Several studies showed that techniques of this kind require a new nomenclature. The 1981 meeting in Paris resulted in publication of a document entitled „An International System for Human Cytogenetic Nomenclature: High-Resolution Banding" (1981) or ISCN (1981).

Applications of chromosome banding in clinical cytogenetics

For more than 20 years, chromosome banding analysis has been the laboratory standard to identify structural and numerical chromosome abnormalities in pre- and post-natal cytogenetics, as well as in cancer diagnosis.

With the application of various staining techniques in individuals with dysmorphic features, several dozen new syndromes have emerged.

Since the late 1960's, the introduction of a broad spectrum of pre-natal tests such as amniocentesis (Steele and Breg, 1966; Valenti et al., 1968), chorionic villi sampling (Simoni, 1983; Kalousek, 1983), and cordocentesis (Daffos, 1992) allowed pre-natal genetic analyses. The rapid proliferation of new techniques in pre-natal diagnosis and the improved resolution of techniques for banding on fetal chromosomes have led to a significant increase in the rate of detection of pre-natally diagnosed chromosome disorders (Milunsky, 1988).

The reliability of a normal pre-natal diagnosis is sometimes restricted by undetected chromosomal mosaicism and maternal contamination. The flask method of amniotic cell culturing (Hoehn et al., 1974; Johnston et al., 1996), based on cytogenetic analysis of at least 20 mitoses, allowed exclusion of 41% of chromosome mosaicism at a 95% confidence level (Hook, 1977; Claussen et al., 1984). The introduction of *in situ* culturing of amniotic fluid (Philip et al., 1974; Tabor et al., 1984) made it possible to verify chromosomal mosaicism detected in chorionic villus sampling (Cheung et al., 1990). By January 2004, clonal chromosomal aberrations identified by various banding techniques had been reported in more than 45,000 human neoplasms (Mitelman et al., 2004 a). At present, almost 500 recurrent balanced neoplasm-associated aberrations have been identified (Mitelman, 2004).

The detection and precise identification of chromosomal aberrations related to genetic diseases and involved in the malignant transformation of tumor cells clearly improves diagnosis and contributes to therapy decisions.

MOLECULAR CYTOGENETICS IN DIAGNOSIS OF CHROMOSOME DISORDERS

Conventional chromosome banding techniques are not sufficient to detect and identify all chromosomal aberrations present in a metaphase. The resolution of classical cytogenetic techniques is limited at best to 4-5 MB, and smaller chromosomal aberrations like ones on small marker chromosomes, subtle translocations, or complex chromosomal rearrangements often remain hidden. These diagnostic problems have

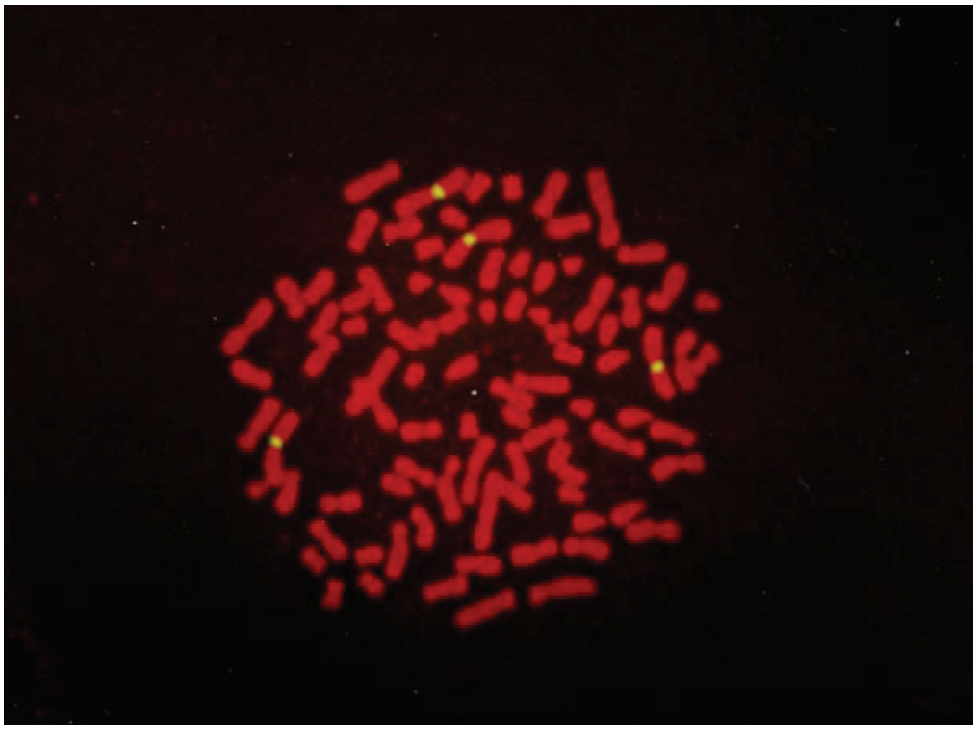

Fig. 2. Metaphase chromosomes of patient with tetraploidy (92, XXXX) after FISH with centromeric probe for chromosome 1 (note 4 signals) (Guc-Scekic et al., 2002).

been overcome in some degree by the introduction of modern molecular cytogenetic techniques such as fluorescence *in situ* hybridization (FISH). Increased application of different FISH techniques resulted in ISCN (1995), which deals with nomenclature for various *in situ* hybridization (ish) applications.

FISH

In this technique, DNA probes are labeled with a fluorescent dye, denatured, and applied to metaphase spreads or interphase nuclei of the patients (Bauman et al., 1980; Langer et al., 1981; Cremer et al., 1988; Pinkel et al., 1988; Jauch et al., 1990; Lichter et al., 1992). The region where hybridization has occurred can be visualized using a fluorescent microscope. In general, FISH has several advantages: firstly, for a number of small and subtle structural rearrangements (routinely in the range of 5-5000 kb), the resolution of FISH is superior to that of classical banding (Riegel et al., 2005). Secondly, FISH can be done independently of the cell cycle as signals can be visualized to intact interphase nuclei.

Using FISH, only a fraction of the genome can be analyzed by region-specific probes (whole chromosome painting probes, probes for sub-chromosomal regions, or gene-specific probes). This means that the FISH technique is mainly used to confirm the presence of already suspected chromosomal aberrations and does not make possible complete karyotype screening or genome wide screening (Fig. 2)

(Guc-Scekic et al., 2002). These limitations have been overcome to some extent by introducing multi-color FISH technologies in diagnosis of chromosomal aberrations.

Multicolor-FISH (M-FISH) and multicolor spectral karyotyping (SKY)

M-FISH technology is based on the simultaneous hybridization of 24 or even more chromosome-specific painting probes labeled with different fluorochromes (Speicher et al., 1996). It provides a multi-color human karyotype in which each pair of chromosomes can be identified on the basis of a unique color and thereby highly characteristic emission spectrum when studied using computer-based image analysis (SKY) (Schrock et al., 1996). This method is useful for detecting subtle deletions,

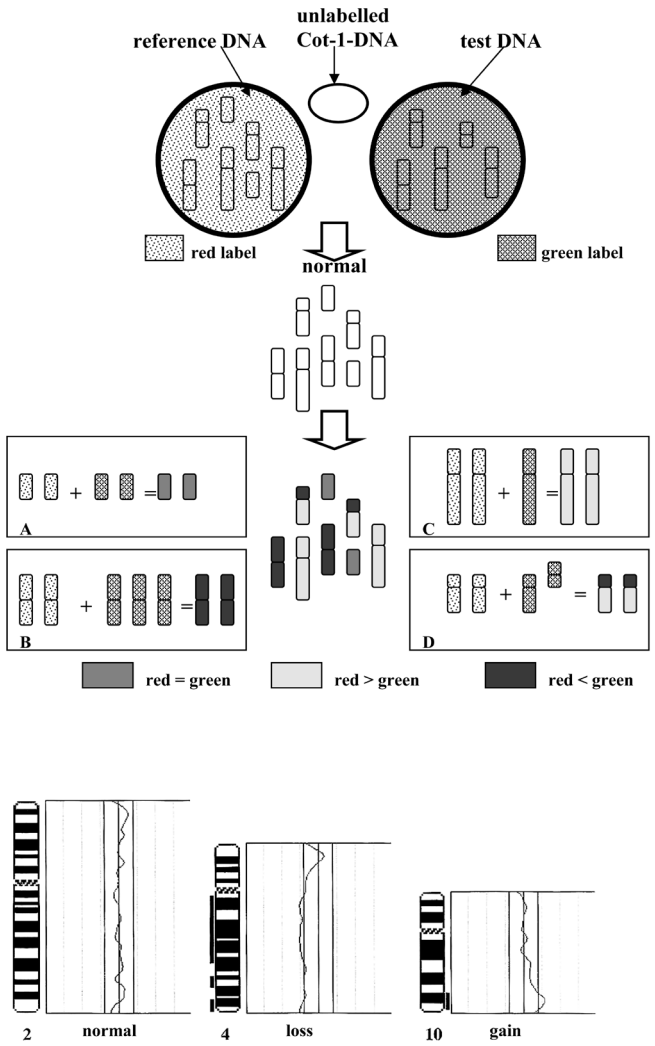

Fig. 3. Schematic representation of the CGH method.

translocations, and supernumerary markers and ring chromosomes. However, the M-FISH method reaches its limits when exact breakpoint localizations of translocations are required, or in the case of very subtle intrachromosomal rearrangements such as interstitial deletions and inversion.

Comparative genomic hybridization (CGH) and microarray CGH

CGH is a new technique by which the entire genome is screened for small chromosomal imbalances. In this technique, the patient's or „test" DNA is labeled with green paint and control (normal) DNA is labeled with a red paint. The two samples are mixed and hybridized to normal methaphase chromosomes and analyzed using a fluorescent microscope. If the test sample contains more DNA from a specific chromosome segment than the control sample, then that region is identified by an increase in the green to red fluorescence ratio. On the other hand, deletion in the test sample is identified by a reduction in the green to to red fluorescent ratio (Kallioniemi et al., 1992; Du Manior et al., 1995; Levy et al., 1998; Turleau et al., 2000) (Fig. 3).

CGH has mostly been used for cancer research, but lately it has also been used in clinical cytogenetics for clarification of complicated rearrangements. However, the use of CGH has so far been restricted by its relatively low sensitivity. Current limits of CGH resolutions are 10 MB for losses and 2 MB for gains. In order to overcome these drawbacks, the microarray CGH method was developed.

In this technique, genomic DNA from the patients is labeled with one fluorescent dye, while a control sample is labeled with a different dye, and these samples are then co-hybridized to an array containing genomic DNA targets. Chromosomal imbalance across the genome can be quantified and positionally defined by analyzing the ratio of fluorescence of the two dyes with the aid of computer software (Solinas-Toldop et al., 1997; Pinkel et al., 1998).

The first-generation array had a coverage of about 1 MB. This resolution has been recently increased to cover the genome at a density of 10-100 kb (Lucito et al., 2003; Ishkanian, 2004), which means that not only single copy changes, but even single copy changes present in mosaics can be accurately detected by this technology (Pollack et al., 1999; Snijders et al., 2001; Fiegler et al., 2003;, Vissers et al., 2003; Schaeffer et al., 2004). Since both genome array CGH and conventional karyotyping based on staining chromosomes aim to identify chromosomal aberrations by positional screening of the genome, it has been proposed that this new technology be called „molecular karyotyping" (Vermeesch et al., 2005).

Implementation of molecular technology in clinical cytogenetics

Nowadays, molecular cytogenetics on human chromosomes is widely used for clinical diagnosis, and it seems likely that molecular karyotyping will rapidly be introduced in cytogenetic laboratories.

FISH technology has proved to be an enormously helpful tool in the precise definition of complex chromosomal aberrations in constitutional cytogenetics and

in the identification of additional materials, where classical banding analysis is often not informative.

FISH with centromeric probes is suitable for making a rapid dignosis of one of the common aneuploidy syndromes (trisomy 13, 18, 21). A set of centromere-specific probes for chromosomes 13, 18, 21, X, and Y can be used for rapid pre-natal diagnosis of the more common numerical chromosomal aberrations. Chromosome-specific single locus probes are particularly useful for identification of tiny sub-microscopic deletions (microdeletion syndromes) and duplications. A complete set of telomeric probes for the 22 autosomes and X and Y chromosomes is used for diagnosis of subtle „cryptic" sub-telomeric translocations and deletions in a significant proportion of children with unexplained mental retardation, but normal G-banded karyotype. FISH with whole chromosome paint probes, M-FISH, and SKY are extremely useful for diagnosis of complex but tiny chromosomal rearrangements; identification of the origin of additional chromosome material such as rings and small supernumerary marker chromosomes (sSMC); and detection of sub-telomeric mutations and tumor karyotype alterations.

The application of CGH and microarray CGH has extended from screening for sub-microscopic chromosomal imbalance in cancer cytogenetics to the detection of any type of gain or loss (resolution of 10-100 kb) of genetic material in dysmorphic and mentally retarded individuals with a normal conventional karyotype (Shaw-Smith et al., 2004; Tyson et al., 2004; Vermeesch et al., 2005).

Molecular cytogenetics on human chromosomes will continue to be influenced by technological improvements. Because of the many advantages of molecular cytogenetics, some voices raise the possibility that molecular cytogenetic analysis will replace classical banding procedures. This will certainly not happen, since none of the newly available approaches can be fully informative on their own. The FISH method reaches its limit when exact breakpoint localizations are required, or in the case of tiny intersticial deletion and inversions. Molecular karyotyping fails to identify balanced translocations and ploidy variations. For these reasons, the cytogeneticist always has to combine the available methods depending on the problem to be solved and use them very carefully for highly specialized goals.

Because of all these limitations, it seems likely that in the foreseeable future, G-banding will remain the gold standard in the genetic diagnostic laboratory, and the starting point for any molecular cytogenetic analysis.

REFERENCES

Arrighi, F.E., and *T. E. Hsu* (1971). Localization of heterochromatin in human chromosomes. *Cytogenetics* **10**, 81-86.

Bauman, J. G. J., Wiegant, J., Borst, P., and *P. van Duijin* (1980). A new method for fluorescence microscopical localization of specific DNA sequences by *in situ* hybridization of fluorochrome-labelled RNA. *Exp. Cell. Res.* **128**, 485-490.

Bobrow, M., and *K. Maden* (1973). The effect of various banding procedures in human chromosomes studied with acridine orange. *Cytogenet. Cell Genet.* **12**, 145-156.

Caspersson, T., Lomakka, G., and *L. Zech* (1972). The 24 fluorescence patterns of human metaphase chromosomes-distinguishing characters and variability. *Hereditas* **67**, 98- 102.

Cheung, S.W., Spitzangel, E., Featherstone, T., and *J. P. Crane* (1990). Exclusion of chromosomal mosaicism in amiotic fluid cultures: Efficacy of in situ versus flask technique. *Pre-nat. Diagn.* **10**, 41-57.

Chicago Conference (1966). Standardization in human cytogenetics. Birth defects. *Original Article Series*, The National Foundation, New York.

Claussen, U., Schafer, H., and *H. J. Trampisch* (1984). Exclusion of chromosomal mosaicism in prenatal diagnosis. *Hum. Genet.* **67**, 23-28.

Cremer, T., Lichter, P., Borden, J., Ward, D. C., and *L. Manuelidis* (1988). Detection of chromosome aberrations in metaphase and interphase tumor cells by *in situ* hybridization using chromosome – specific whole chromosome paint probes. *Hum. Genet.* **80**, 235-246.

Daffos, F. (1991). Fetal blood sampling, In: *The Unborn Patients* (Eds. M. R. Harrison, M. S. Golbus, and R. A. Filly). Saunders, Philadelphia.

De Grouchy, J., and *C. Turleau* (1984). *Clinical Atlas of Human Chromosomes*, Second Edition. John Wiley, Chichester.

Denver Conference (1960). A proposed standard system of nomenclature of human mitotic chromosomes. *Lancet* **1**, 1063-1065.

Drets, M. E., and *M. W. Shaw* (1971). Specific banding patterns of human chromosomes. *Proc. Natl. Acad. Sci. USA* **68**, 2073-2077.

Du Manoir, S., Schrock, E., Bentz, M., Speicher, M. R., Joos, S., Ried, T., Lichter, P., and *T. Cremer* (1995). Quantitative analysis of comparative genomic hybridization. *Cytometry* **19**, 27-41.

Dutrilleaux, B., and *J. Lejeune* (1971). Sur une nouvelle technique d'analyse du caryotype humain. *C. R. Acad. Sci. (D) (Paris)* **272**, 2638-2640.

Fiegler, H., Carr, P., and *E. J. Douglas* (2003). DNA microarrays for comparative genomic hybridization based on DOP.PCR amplification of BAC and PAC clones. *Genes Chromosomes Cancer* **36**, 361-374.

Goodpasture, C., and *S. E. Bloom* (1975). Visualization of nucleolar organizer regions III. Mammalian chromosomes using silver staining. *Chromosoma* **53**, 37-50.

Guć-Šćekić, M., Milasin, J., Stevanović, M., Stojanov, L., and *M. Djordjević* (2002.) Tetraploiidy in a 26-month-old girl (cytogenetic and molecular studies). *Clinical Genetics* 61 (1), 62-65.

Hoehn, H., Byant, E. M. Karp, L.E., and *G. M. Martin* (1974). Cultivated cells from dignostic amniocentesis in second trimester pregnancies. I. Clonal morphology and growth potential. *Pediatr. Res.* **8**, 746-754.

Hook, E. B. (1977). Exclusion of chromosomal mosaicism: tables of 90%, 95% and 99% confidence limits and comments on use. *A. J. Hum. Genet.* **29**, 94-97.

ISCN (1978) (1978). An international system for human cytogenetic nomenclature. *Cytogenet. Cell Genet.* **21**, 309-404.

ISCN (1981) (1981). An international system for human cytogenetic nomenclature: high resolution banding. *Cytogenet. Cell Genet.* **31**, 1-23.

ISCN (1995) (1995). *An International System for Human Cytogenetic Nomenclature* (Ed. F. Mitelman). Karger, Basel.

Iskanian, A., Malloff, C.A., and *S. K. Watson* (2004). A tiling resolution DNA microarray with complete coverage of the human genome. *Nat. Genet.* **36**, 299-303.

Jacobs, P. A., Browne, C., Gregson, N., Joyce, C., and *H. White* (1992). Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. *J. Med. Genet.* **29**, 103-108.

Jauch, A., Daumer, C., Lichter, P., Murken, J., Schroede–Kurth, T., and *T. Cremer* (1990). Chromosomal *in situ* suppression hybridization of human genosomes and autosomes and its use in clinical cytogenetics. *Hum. Genet.* **85**, 145-150.

Johnson, J. A. M., Wilson, R. D., Winsor, E. J. T., Singer J., Dansereau, J., and *D.K. Kalousek* (1996). The early amniocentesis study: a randomized clinical trial of early amniocentesis versus midtrimester amniocentesis. *Fetal Diagn. Ther.* **11**, 85-93.

Kallionemi, A., Kallionemi, O. P., and *D. Sudar* (1992). Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* **258**, 818-21.

Kalousek, D. K. (1983). Chromosomal mosaicism confined to the placenta in human conceptions. *Science* **221**, 665-667.

Langer, P. R., Waldrop, A. A., and *D. A. Ward* (1981). Enzymatic synthesis of biotin labeled polynucleotides: novel nucleic acid affinity probes. *Proc. Natl. Acad. Sci.* USA **78**, 6633-6637.

Latt, S.A., and *J. Wohhleb* (1975). Optical studies of the interaction of 33258 Hochst with DNA, chromatin and metaphase chromosomes. *Chromosoma* **52**, 297-316.

Lejeune, J. (1959). Premier example d'aberration autosomique humaine. *Ann. Génét.* **11**, 116-119.

Levy, B., Dunn, T. M., Kaffe, S., Kardon, N., and *K. Hirschhorn* (1998). Clinical application of comparative genomic hybridization. *Genet. Med.* 4-12.

Lichter, P., and *T. Cremer* (1992). Chromosome analysis by non-isotopic *in situ* hibridization. In: *Human Cytogenetics. A Practical Approach, Volume I,* (Eds. D. N. Rooney and B. H. Czepujkowski), 157-190, IRL Press, Oxford, New York, Tokyo.

Lucito, R., Healy, J., and *J. Alexander* (2003). Representational oligonucleotide microarray analysis: a high-resolution method to detect genome copy number variation. *Genome Res.* **13**, 2291-2305.

Matsui, S.I., and *M. Sasaki* (1973). Differential staining of nucleolus organisers in mammalian chromosomes. *Nature* **246**, 148-150.

Milunsky, A. (1988). *Genetic Disorders and the Fetus*. Plenum Press, New York.

Mitelman, F. (1991). *Catalog of Aberrations in Cancer*, Fourth Edition. Wiley-Liss Inc., New York.

Mitelman, F., Johansson, B., and *F. Meretens* (2004 a). *Mitelman Database of Chromosome Aberrations in Cancer.* http://cgap.nci.nih.gov/Chromosomes/Mitelman

Mitelman, F., Johansson, B., and *F. Meretens* (2004 b). Fusion genes and rearranged genes as a linear function of chromosome aberrations in cancer. *Nat. Genet.* **36**, 331-334.

Pai, G. S., and *G. H. Thomas* (1980). A new R banding technique in clinical cytogenetics. *Hum. Genet.* **54**, 41-45.

Paris Conference (1971) (1972). Standardization in Human Cytogenetics. *Cytogenet.* **11**, 313-362.

Philip, J., Bang, J., Hahnemann, N., Mikkelsen, M., Niebuhr, E., Rebbe, H., and *J. Weber* (1974). Chromosomal analysis of fetuses in risck pregnancies. *Acta Obstet. Gynec. Scand.* **53**, 9-14.

Pinkel, D., Lanfegent, J., Collins, C., Fuscoe, J., Segraves, R., Lucas, J., and *J. W. Gray* (1988). Fluorescence *in situ* hybridization with human chromosome – specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc. Natl. Acad. Sci.* USA **85**, 9138-9142.

Pinkel, D., Segraves, R., and *D. Sudar* (1998). High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat. Genet.* **20**, 297-211.

Pollack, J. R., Perou, C. M., and *A. A. Alizadeh* (1999). Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat. Genet.* **23**, 41-46.

Riegel, M., Hargreaves, P., Baumer, A., Guc-Scekic, M., Ignjatović, M . and *A. Schinzel* (2005). Unbalanced 18q/21q translocation in a patient previously reported as monosomy 21. *Eur. J. Med. Genet.* **48 (2)**, 167-74

Schaeffer, A.J., Chung, J., Heretis, K., Wong, A., Ledbetter, D.H., and *M. C. Lese* (2004). Comparative genomic hybridization- array analysis enhances the detection of aneuploidies and sub-microscopic imbalances in spontaneous miscarriages. *Am. J. Hum. Genet.* **74**, 1168-1174.

Schinzel, A. (1994). *Human Cytogenetics Database*. Oxford University Press, Oxford.

Schrock, E., du Manoir, S., Veldman, T., Schoell, B., Wienberg, J., Ferguson-Smith,M. A., Ledbetter, D.H., Bar-AM, I. Soenksen, D., Garini, Y., and *T. Ried* (1996). Multicolor spectral karyotyping

of human chromosomes. *Science* **273**, 494-497.

Seabright, M. (1991). A rapid banding technique for human chromosomes. *Lancet* **2**, 971-972.

Shaw-Smith, C., Redon, R., and *L. Rickman* (2004). Microarray based comparative genomic hybridisation (array-CGH) detects sub-microscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *J. Med. Genet.* **41**, 241-248.

Simoni, G., Brambati, B., Danesino, C., Rosella, F., Terzoli, G. L., Ferrari, M., and *M. Fraccaro* (1983). Efficient direct chromosome amalysis and enzyme determinations from chorionic villi samples in the first trimester of pregnancy. *Hum. Genet.* **63**, 349-357.

Sniders, A. M., Novak, N., and *R. Segraves* (2001). Assembly of microarrays for genome-wide measurment of DNA copy number. *Nat. Genet.* **29**, 263-264.

Solainas-Toledo, S., Lampel, S., and *S. Stilgenbauer* (1997). Matrix- based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromosomes Cancer* **20**, 399-407.

Speicher, M.R., Ballard, S.G., and *D. C. Ward* (1996 a). Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat. Genet.* **12**, 368-375.

Speicher, M,R., Ballard, S.G., and *D. C. Ward* (1996 b). Computer image analysis of combinatoral multi-fluor FISH. *Bioimaging* **4**, 52-64.

Steele, M,W., and *W. R. Breg* (1966). Chromosome anslysis of human amniotic fluid cells. *Lancet* **1**, 383-385.

Tabor, A., Lind, A.M., and *J. Philip* (1984). A culture vessel for amniotic fluid cells allowing faster preparation of chromosome slides. *Prenat. Diagn.* **4**, 451-455.

Tjio, J. H., and *A. Levan* (1956). The chromosome number of man. Hereditas **42**, 1-6.

Turleau, C., Lapierre, J.M., Joly, G., Prieur, O., Raoul, O., and *M. C. de Blois* (2000). CGH as a routine procedure in a clinical cytogenetic laboratory: a one-year assessment. *Am. J. Hum. Genet. Suppl.* **67,** 152.

Turnpenny, P., and *S. Ellard* (2005). Emery's Ellements of Medical Genetics, 12th Edition, Elsevier-Churchill-Livingstone, London.

Tyson, C., McGillivary, B., Chijiwwa, C., and *E. Rajcan-Separovic* (2004). Elucidation of a criptic interstitial 7q31.3 deletion in a patient with a language disorder and mild mental retardation by array CGH. *Am. J. Med. Genet.* **129A**, 254-260.

Valenty, C. (1968). Chromosome study of throphoblastic tissue. *Am. J. Obstet. Gynecol.* **92**, 211-214.

Vermeesh, J.R., Debiec-Rychter, M., Melotte, C., Maas, N., Fryns, J,P., and *K. Devriendt* (2005). Molecular karyotyping:towards improved pre-and postanatal diagnosis. *ECA Newsletter* **15**, 9-15.

Vissers, L. E., de Vries, B. B., and *K. Osoegawa* (2003). Array- based comparative genomic hybridization for the genome-wide detection of sub-microscopic chromosomal abnormalities. *Am. J. Hum. Genet.* **73**, 1261-1270.

Yunis, J. J. (1976). High resolution of human chromosomes. *Science* **191**, 1268-1270.