

Historical prospective of human cytogenetics: from microscope to microarray

Dominique F.C.M. Smeets*

Department of Human Genetics, University Medical Center, Nijmegen, The Netherlands

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Abstract

After the fundamental discovery in 1956 that normal human cells contain 46 chromosomes, clinical cytogenetics was born and studies into the relation of chromosomal defects and disease could begin. Although many technical advances have been made over this long period, including the introduction of molecular techniques, until now, all cytogenetic studies have been performed through regular microscopes, which was throughout the years the most important equipment of a cytogenetic laboratory. However, recently a new technique has been introduced based on comparative genomic hybridization on an array of thousands of different probes (array-CGH). This technique enables an increase in the sensitivity of detecting chromosomal aberrations far beyond the detection limit of regular banding techniques. Furthermore, it gives us the possibility to detect genomic changes in malignant cells in cases where aberrations are too complex to study or when chromosomes are not available at all. Cytogenetic laboratories are now challenged to introduce and incorporate this new application next to the various well-established microscopical techniques to provide optimal diagnostic services.

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The early days of human cytogenetics

Cytogenetics is the study of structure, function, and evolution of chromosomes and it was at the end of the 19th century that the first tentative steps were taken into the field of *human* cytogenetics. Flemming published in 1882 the first drawn illustrations of human chromosomes [40] and in 1888 the term “chromosome” (Greek for “stained body”) was coined by Waldeyer [42,43]. Only a couple of years later, it was already anticipated that chromosomes probably constitute the physical basis of heredity.

However, cytogenetic studies were tough those days. It was not yet possible to culture mammalian cells nor to produce any quality slides which enabled a reproducible count of the number of chromosomes. Researchers had to deal with biopsies (for instance from the testis), tissue sections or squashed tumor cell samples. In this light, it

was not strange that the number of chromosomes in human cells conflicting results was reported. Von Winiwarter [1] counted a different number in males ($n = 47$) as in females ($n = 48$) and Painter published in 1923 several 48 based on a study of meiotic chromosomes in several testis biopsies taken from incarcerated, castrated males who had been sentenced to death [41]. In fact, this figure of 48 became generally accepted in the years thereafter and in the end was taken as a given fact by most of the researchers for over three decades, since they were not equipped to prove otherwise. It was therefore for good reason that Hsu [2] in his very nice overview of these early days of human cytogenetics refers to this period as “the dark ages” because everybody just copied these first results without adding any new data (reviewed also by Makino [3]).

Fortunately, times changed from the early 1950s onwards. Several new exciting discoveries were made which in the end culminated in the discovery of the correct number of chromosomes in man. For instance, more and more successes were claimed in culturing various types of mammalian cells, which enabled a relatively easy access to dividing cells. Still, it remained cumbersome to get nice slides with well spread

* Department of Human Genetics, University Medical Center Nijmegen, PO Box 9101, 6500 HB, Nijmegen, The Netherlands. Fax: +31-243542151.

E-mail address: D.Smeets@antrg.umcn.nl.

chromosomes which would make it possible to count them easily. This remained the case until Hsu [4] described the so-called hypotonic shock. He found out that cells that had been kept for several minutes in a hypotonic salt solution instead of an isotonic one before fixation gave a much better spreading of the chromosomes. This crucial finding was a beautiful example of serendipity since one of his co-workers had accidentally applied ordinary water instead of a salt solution for washing the cells. Yet, another important finding was the addition of colchicine to cell cultures, which destroys the mitotic spindle and captures the cells in their metaphase stage [5,6]. Subsequently, the cells are not able to finish mitosis and an increasing number of metaphases become available for microscopic studies.

These developments culminated in the year 1955 in Lund (Sweden) where Tjio, who had worked at the laboratory of Ford, and Levan who had worked with Hsu, performed several experiments with cultured embryonal lung cells. Combining the latest developments, they were able to make beautiful chromosome slides from these cells which unequivocally proved that the number of chromosomes in man was 46. Nevertheless, they remained very cautious when publishing their results: "...we do not wish to generalize our present finding into a statement that the chromosome number of man is $2n = 46$, but it is hard to avoid the conclusion that this would be the most natural explanation of our observations" [7]. Fortunately, at the end of the same year, their conclusion was independently confirmed in studies of human spermatocytes by Ford and Hamerton [5,6]. These papers in fact paved the way for the start of a new era of clinical cytogenetics. Although the chromosomes showed only few details, they could be arranged in eight different groups (A–G, and the sex chromosomes) (Fig. 1) based on their size and

location of the primary constriction (centromere). This enabled an easy counting of the chromosomes and in the following years, several numerical chromosome aberrations were reported like trisomy 21 in Down syndrome [8], 45,X in Turner syndrome [9], 47,XXY in Klinefelter syndrome [10], trisomy 13 [11], and trisomy 18 [12]. Indeed, the first acquired (not constitutional) chromosome abnormality (the "Philadelphia chromosome") was recognized in bone marrow cells of a patient suffering from chronic myeloid leukemia [13]. However, it took 13 years before it became apparent that this was not a simple deletion but the result of a reciprocal translocation between the chromosomes 9 and 22 [14]. After almost another 13 years, it was discovered that this translocation resulted in a new fusion gene directly responsible for the leukemic development of the cells [15].

In 1966, Steele and Breg [16] reported that cells cultured from amniotic fluid could be used to determine the chromosome content of the fetus. This is the technique that is still most widely used for prenatal chromosome studies.

Based on the increasing success in culturing various types of cells, also many studies were conducted in establishing the chromosome content of human abortions, and miscarriages and soon it became apparent that many of them (over 50% of abortions) showed numerical aberrations, resulting in a tetra- or triploidy, a trisomy (e.g., chromosomes 13, 18, 21, and often 16) or monosomy (45,X) [17,18]. Obviously, as compared to other mammals, in humans, many errors are made during meiosis and around fertilization. The reasoning behind this phenomenon is still under debate although one of the factors, which may play an important role herein is the relatively advanced age at which humans reproduce as compared to other species. That age plays a major role is clearly shown by the greater chance of

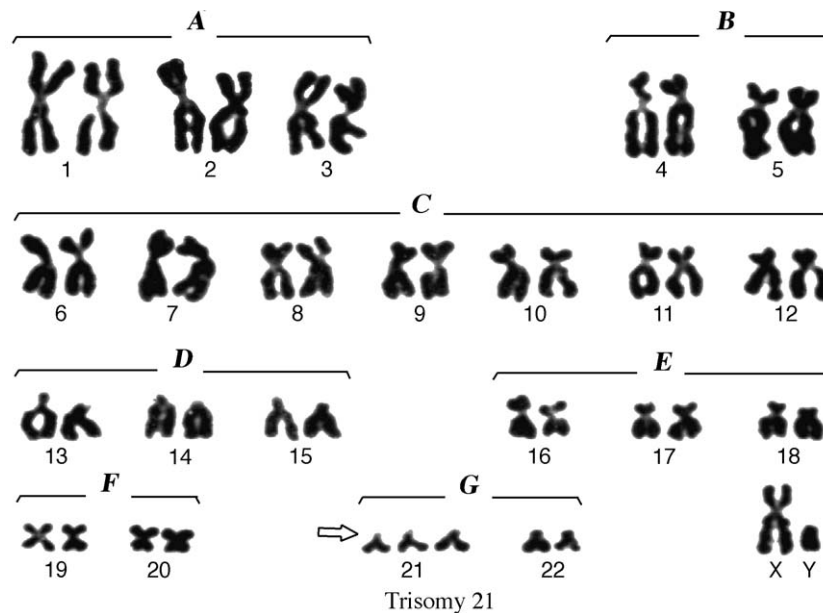


Fig. 1. Original karyotype of a trisomy 21 (Down syndrome) after "solid" Giemsa staining. Since it is impossible to recognize all individual chromosomes, they are subdivided in several groups (A–G and sex chromosomes) based on their total length and location of the centromere.

getting unbalanced offspring with increasing age of the mother, rising from 1 in 250 at the age of 36 to 1 in 10 at the age of 45. Nevertheless, also in young women, one in every seven recognized pregnancies results in an abortion often showing a chromosomal abnormality, indicating that at all ages, the number of meiotic failures in humans is relatively high [17–19].

Chromosome banding

Until the early 1970s, all cytogenetic studies were performed on “solid” stained chromosomes which hampered unequivocal identification of individual chromosomes and the detection of most of the structural aberrations. Therefore, many efforts were made to develop a technique for a clear discrimination between the various chromosome pairs. Caspersson et al. [20,21] was the first who really could show a banding pattern, which enabled recognition of every single chromosome. This banding pattern was based on a fluorescent staining technique with quinacrine and clearly visible in fluorescent microscopy. However, fluorescence intensity quickly quenched which made the technique less optimal for routine studies of patients suspected of having a chromosome aberration. Hence, several other banding techniques were developed like G-, R-, C-, and NOR-banding each having their own specific properties and applications [22]. For routine use in a clinical setting, the G-banding technique based on the application of trypsin followed by Giemsa staining became the most accepted method worldwide [23] (Fig. 2). Implementation of these banding techniques accompanied by easy culture methods of peripheral lymphocytes from only few milliliters of whole blood [24,25] resulted in a significant increase in the number of routine chromosome studies. Since the banding pattern enabled the detection of various structural

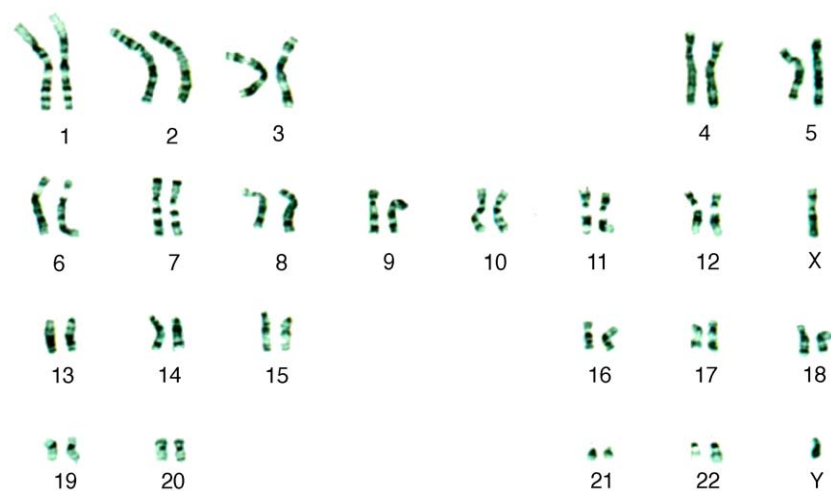


Fig. 2. Karyotype of a normal male. The chromosomes show a banding pattern after a treatment of trypsin and subsequent staining with Giemsa which enables a relatively easy identification of every single chromosome.

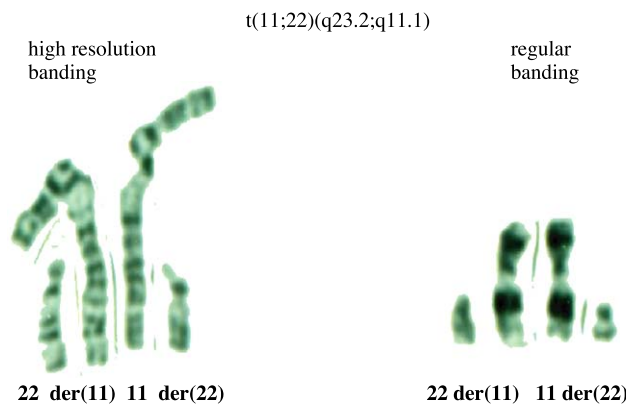


Fig. 3. Balanced translocation between chromosomes 11 and 22. At the right side of the picture are the chromosomes 11 and 22 of a normal metaphase cell, while the left side shows high resolution chromosomes from a prometaphase. The aberrant chromosomes are presented as der(11) and der(22).

aberrations like translocations, inversions, deletions, and duplications next to the already well-known numerical aberrations, not only potentially unbalanced cases (patients) could be studied but also healthy individuals as possible carriers of a balanced aberration. For instance, healthy family members of already known carriers and couples suffering from repetitive spontaneous abortions were from now on cytogenetically investigated.

Unfortunately, despite the introduction of a banding pattern, resolution of chromosome studies remained relatively limited with an approximate count of 500 bands per haploid genome (resolution \approx 6 million base pairs \approx 50 genes per band). This was improved by the development of so-called high-resolution banding by Yunis [26]. By synchronizing lymphocyte cultures, he significantly increased the number of cells being in pro-metaphase or even pro-phase stage instead of metaphase. In these much longer

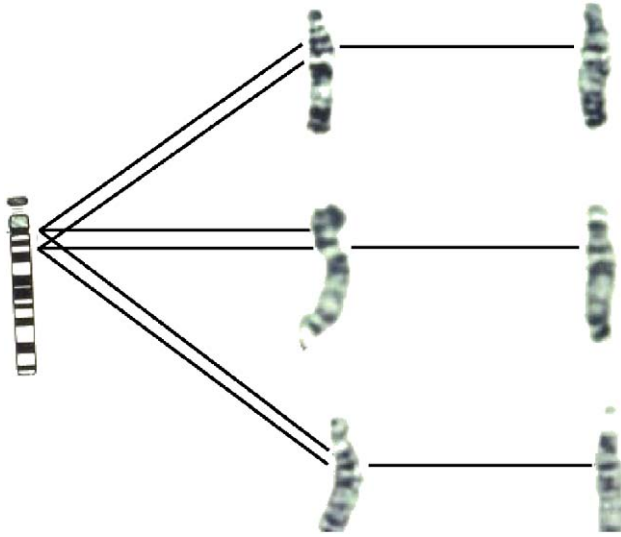


Fig. 4. High resolution banding of chromosome 15 from 3 different patients with Prader Willi syndrome. From left to right, the ideogram of a normal #15, than in the middle the normal #15 while on the right side the #15 with a deletion of the most proximal band is shown. Both the normal and abnormal #15 are from the same cell.

chromosomes, regular metaphase bands appeared to split-up in many sub-bands, thus increasing resolution from 500 to over 1000 bands in a haploid genome. This made not only a much more precise description of already known chromosomal aberrations feasible (Fig. 3) but facilitated also the detection of thus far unnoticed subtle aberrations, like duplications or deletions. By applying this technique, several already well-known clinical syndromes could be linked to small chromosome aberrations and the concept of the micro-deletion or contiguous gene syndrome was born [27]. Well-known examples are Prader Willi and Angelman syndrome with a deletion at the proximal long arm of chromosome 15 (Fig. 4), Smith-Magenis and Miller-Dieker syndrome with (different) deletions in the short arm of chromosome 17, and DiGeorge/Velo Cardio Facial (VCF) syndrome with deletions in the long arm of chromosome 22.

Start of molecular cytogenetics

Despite the development of high-resolution chromosome banding, still in numerous patients showing clear clinical signs of one of the above-mentioned syndromes, no aberrations were visible at the cytogenetic level. It was therefore important that a completely new technique was introduced into the field of clinical cytogenetics: fluorescence in situ hybridization (FISH) (Fig. 5) ([28,29 reviewed by Trask [30]). Molecular cytogenetics was born, and once more, the big gap between cytogenetics and molecular studies became somewhat smaller. This technique evolved from the already well-known radioactive labeling procedures of probes, for instance, used in mapping studies. However, this type of

labeling was very time-consuming and difficult to perform and therefore not adequate for regular use in a clinical laboratory setting. On the other hand, FISH became rather quickly available for routine use and was applicable for various situations such as solving complex aberrations, detection of very small submicroscopic deletions, and even studies on interphase cells. At this moment, thousands and thousands of different probes from all over the genome are available by which certain parts of chromosomes (e.g., centromeres), whole chromosomes, and regions susceptible for the arisal of deletions and/or duplications can be specifically stained (Figs. 6a–c). By making use of various combinations and concentrations of fluorescent dyes, it is even possible to give every single chromosome a different color (spectral karyotyping = SKY; Fig. 6d), which can be of particular use when dealing with complex aberrations often associated with various types of solid tumors.

As a spin-off of the Human Genome Project (HUGO), more and more probes from cloned and mapped segments of the human genome (cosmids, PACS, BACS, and YACS) became available for diagnostic purposes (e.g., BAC Resource Consortium, Cheung et al. [31]).

Since in many cases, imbalances may be the result of an (small) unbalanced translocation between two chromosomes, several studies were performed with FISH using probes of the tips of the chromosomes (subtelomeric probes). Soon it appeared that around 5% of patients with mental retardation indeed showed a deletion or duplication of a certain telomere [32–34]. Next to this, the awareness grew that there are probably much more submicroscopical aberrations (i.e., deletions and duplications) present at various sites within the human genome, in particular in patients suffering from mental retardation and/or multiple congenital anomalies, than what was assumed thus far. However, since for FISH investigations all probes have to be hybridized and afterwards microscopically analyzed, such studies are very time-consuming, difficult to automate, and can therefore be applied for only a limited number of probes. Hence, again, new techniques were explored and finally an array based on comparative genomic hybridization was developed [35–37]. These kinds of arrays can be regarded as a very large reversed FISH experiment. Instead of hybridizing a labeled probe to human chromosomes on a slide, we now have the potential to print thousands of different and well-characterized probes on a glass slide. Subsequently, complete isolated and fragmented DNA from the patient is labeled in a certain color and mixed with exactly the same amount of DNA of a normal control (or a mix of controls) which is labeled in a different color. This DNA mix is then hybridized to the denaturated probe DNA on the glass slide. After several washing steps, the fluorescence pattern of each spot can be analyzed and the ratio of test (patient) over reference (control) is measured (Fig. 7a).

As a first experiment, we set out to assemble a telomere array containing two different probes of the tip of every chromosome arm (Fig. 7b). On this array, several patients

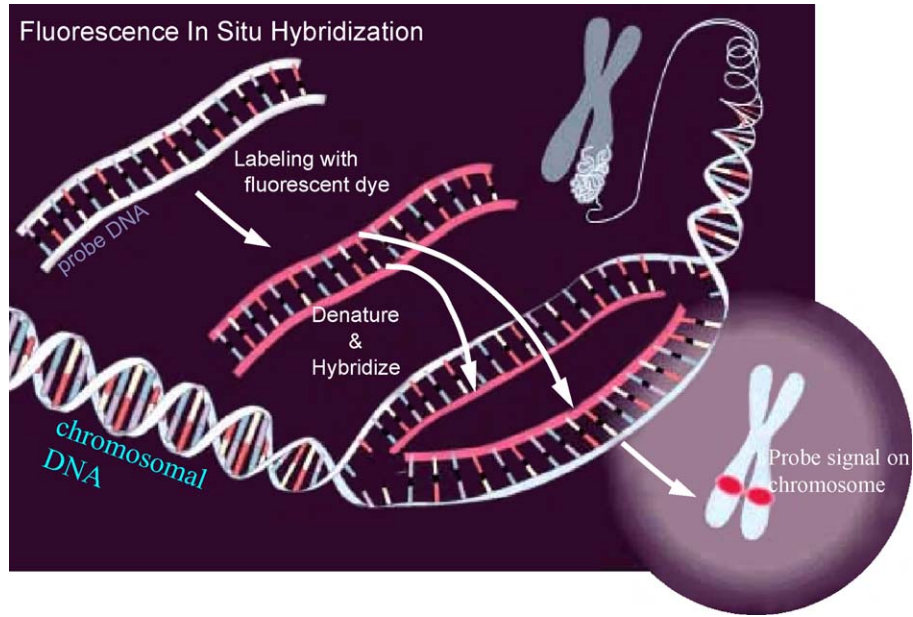


Fig. 5. Fluorescence in situ hybridization: the chromosomal DNA is present on the slide as metaphase chromosomes while the labeled probe DNA is added onto the slide for hybridization for a period of several hours (overnight). After several washing steps to remove the probe DNA, which is not firmly bound to the chromosomes, the slides are studied in fluorescence microscopy after the chromosomal DNA is counterstained with DAPI for recognition of the chromosomes.

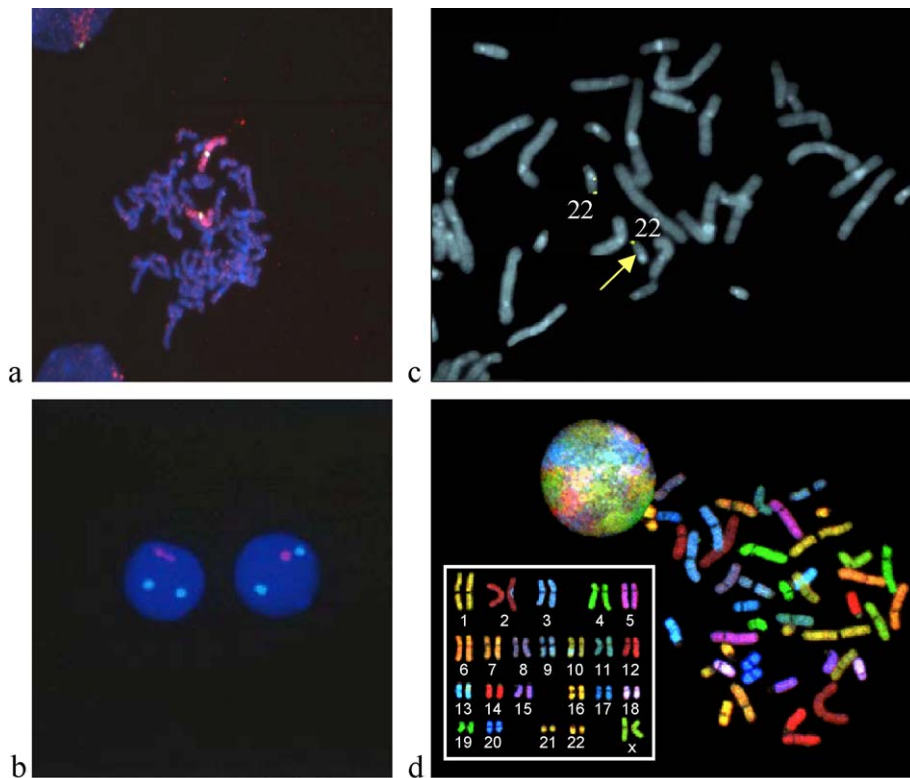


Fig. 6. Various applications of FISH. (a) Simultaneous chromosome paints of the X-chromosomes (pink) and their centromeres (yellow). (b) Interphase FISH with probes specific for the Y-chromosome (red) and the X-chromosome centromere (blue) on uncultured amniocytes of a fetus with a 47,XXY karyotype. (c) Detection of a submicroscopic deletion in the long arm of a chromosome 22 in a patient with DiGeorge syndrome. The normal chromosome 22 shows two signals (one from the DiGeorge region and one from the tip of the long arm as a control signal), while at the second chromosome 22, only the control signal appears to be present since the DiGeorge region is absent (arrow). (d) Spectral karyotyping (SKY) of the complete chromosome complement, where all chromosome pairs have their own (artificial) color.

were analyzed with already well-known cytogenetically established deletions to check whether the technique works. One of these cases is shown in Figs. 7c (karyotype) and d. Indeed, in all tested patients, the deletions were clearly shown [38].

A big advantage of this new microarray technique is the possibility to automate large parts of the procedure and to upgrade the number of probes significantly. The next step was therefore to establish a much larger array chip that included 3500 BAC clones evenly spaced at about every megabase from the human BAC/PAC resources [31]. All these clones were single cultured and FISH verified (correct location of the clone). After amplification, the DNA of all clones was spotted onto a glass slide in triplicate. In the first experiments, again, we used DNA of several patients with well-known deletions to validate the procedure (Fig. 8).

Thereafter, 20 selected patients who were all mentally retarded with some additional clinical features were analyzed, and in five of them, aberrations were detected, including a relatively large deletion in the long arm of chromosome 7 corresponding to Williams syndrome. Subsequent analysis of the parents of the patients, however, revealed that only two aberrations were de novo and therefore probably directly related to the clinical symptoms, whereas the three others appeared to be present in one of the parents and apparently constitute polymorphisms [39]. This is an intriguing finding because it indicates that the human genome shows a much larger plasticity and as such contains much more polymorphisms than was ever held possible.

In conclusion, the CGH-array technique will soon become the method of choice in analyzing patients with mental retardation and/or congenital anomalies because

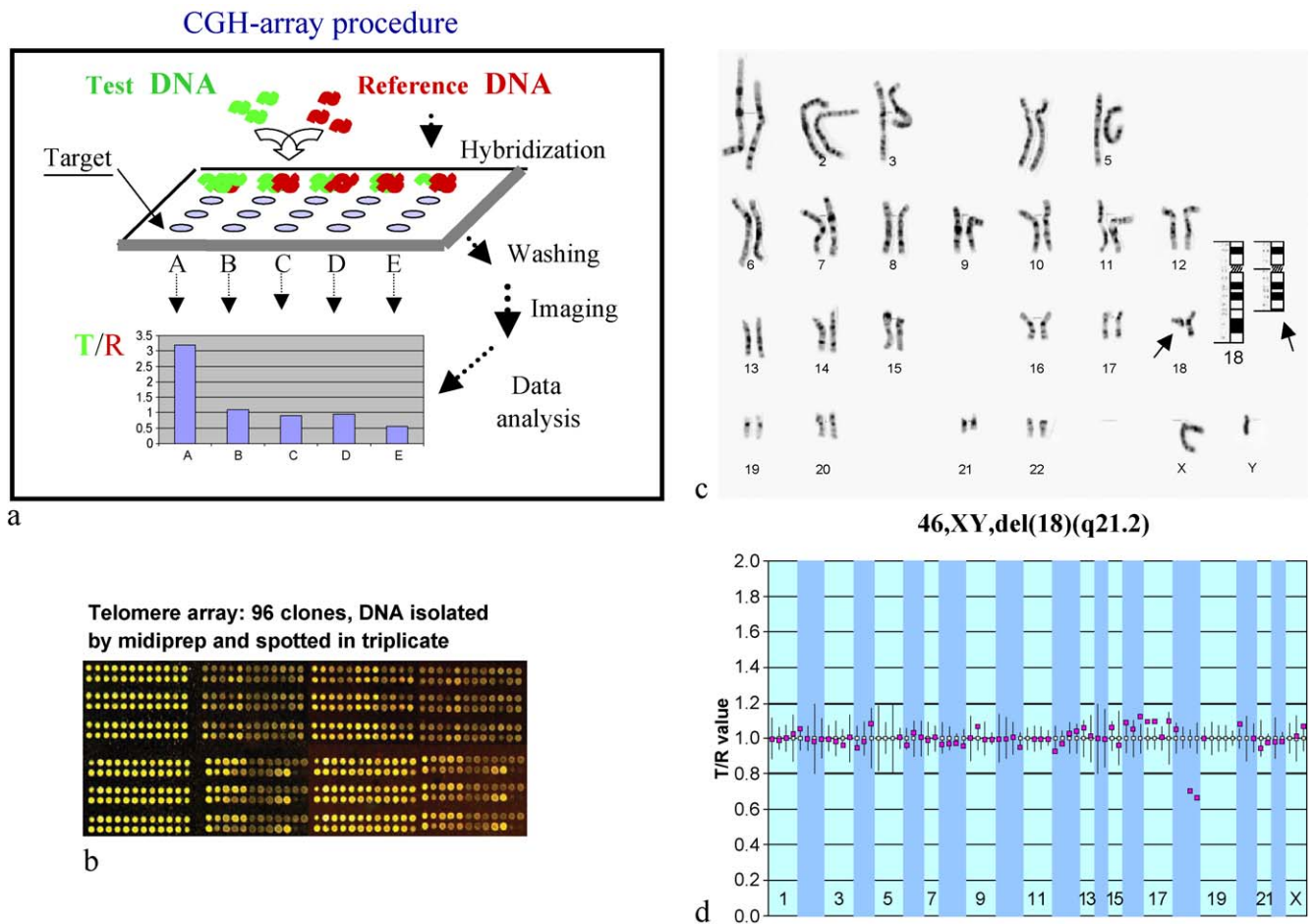


Fig. 7. CGH-array procedure. (a) Test DNA (patient DNA) and reference DNA (control DNA) are labeled in different colors and mixed in equal concentrations. Then, this mixture is hybridized to the probe DNA (target) that has been printed in different spots onto a glass slide. Every spot comprises DNA from a single well-characterized BAC or PAC from a known location within our genome. In case the patient has a trisomy (for instance for chromosome 21), then relatively more chromosome 21 DNA (green) from the patient will be present relative to that of the control (red), and after hybridization, the measured signals at the spots of clones derived from chromosome 21 DNA look more “greenish”. If on the other hand, the patients show a deletion, the signal from the spots of clones that fall into the deletion will give a more “reddish” signal (relatively more DNA from the control than from the patient will hybridize). Since thousands of different DNA spots can be printed on one single slide, the resolution can be very high. (b) Example of a relatively small array spotted from various subtelomeric clones. (c) Karyotype of a patient with a deletion of part of the long arm of chromosome 18 (arrow) who was analyzed on the telomere array. (d) Array results of the above-mentioned patient. Clearly visible are the two signals derived from clones located at the tip of the long arm of chromosome 18 (lane 18) which fall below the boundaries of the Test over Reference value (T/R) which are set at 0.8 and 1.2 (normal spreading of the signals).

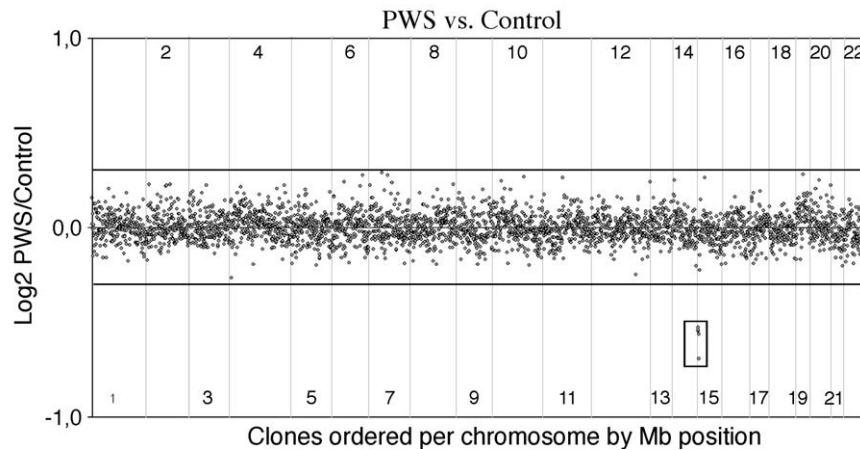


Fig. 8. Array-CGH result of a patient with Prader Willi syndrome and a small deletion in chromosome 15 against a normal control. The array contained 3500 different human autosomal FISH-verified clones. Every vertical lane resembles a single chromosome from chromosomes 1 to 22 (from left to right). The two dark horizontal lines demarcate the normal variation of the spot signal. In lane 15 (chromosome 15), four clones (within the box) clearly fall outside these boundaries thus pinpointing the deletion in chromosome 15 (see also Vissers et al. [39]).

the standard cytogenetic resolution of approximately 500 bands per haploid genome can now be increased to at least 3500 clones (± 7 clones per chromosome band) evenly distributed over the genome and this certainly is not the end. Already, the complete human genome is available in the high-resolution BAC re-arrayed clone set (the “32 k set”). This clone set has been generated by scientists at the Genome Sciences Center in Vancouver in collaboration with scientists from the Children’s Hospital Oakland Research Institute and our own group (see <http://bacpac.chori.org/pHumanMinSet.htm> and <http://www.bcgsc.ca/lab/mapping/bacrearray/human/>). It comprises 32,000 overlapping BAC clones, which can all be used in CGH-array experiments. This implies that deletions as well as duplications with a size of at least 200 kb will probably be readily detected throughout the genome. However, in many aberrant cases, it will be necessary to study both parents as well, since many duplications and deletions will appear to be harmless polymorphisms. Additionally, these techniques will tell us much more about changes and variation within the human genome.

Although cytogenetics is moving into the direction of molecular approaches instead of microscopy, these developments by no means imply that the “old fashioned” banding techniques which we have practiced for over 30 years now will disappear from the laboratories. Still regular karyotyping remains the single (and simple) technique to obtain a quick overview of the complete human genome. Moreover, and even of greater importance is the fact that balanced aberrations like chromosome translocations, inversions, insertions, etc. can be visualized only through the microscope and not by other techniques simply because these detect only unbalanced situations. Therefore, the world of cytogeneticists will not be constraint; on the contrary, banding techniques can now be combined by multicolor FISH and state-of-the-art molecular techniques which will

add yet another dimension to improve diagnostic services as well as research opportunities.

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References

- [1] Von Winiwarter H. Études sur la spermatogenèse humaine: I. Cellule de sertoli: II. Hétérochromome et mitoses de l’épithelium seminal. *Arch Biol* 1912;27:91–189.
- [2] Hsu TC. Human and mammalian cytogenetics: an historical perspective. Heidelberg: Springer-Verlag; 1979.
- [3] Makino S. Human chromosomes. Tokyo: Igaku Shoin; 1975.
- [4] Hsu TC. Mammalian chromosomes in vitro: I. The karyotype of man. *J Hered* 1952;43:167–72.
- [5] Ford CE, Hamerton JL. A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. *Stain Technol* 1956;31:247.
- [6] Ford CE, Hamerton JL. The chromosomes of man. *Nature* 1956; 178:1020–3.
- [7] Tjio JH, Levan A. The chromosome number in man. *Hereditas* 1956; 42:1–6.
- [8] Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongoliens. *Comptes Rendus* 1959;248:1721–2.
- [9] Ford CE, Jones KW, Polani PE, et al. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner’s syndrome). *Lancet* 1959;i: 711–3.
- [10] Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature* 1959;183:302–3.
- [11] Patau K, Smith DW, Therman E, et al. Multiple congenital anomaly caused by an extra autosome. *Lancet* 1960;i:790–3.
- [12] Edwards JH, Harnden DG, Cameron AH, et al. A new trisomic syndrome. *Lancet* 1960;i:787–90.

- [13] Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Science* 1960;132:1497–501.
- [14] Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973;243:290–3.
- [15] Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G. Structural organization of the bcr gene and its role in the Ph¹ translocation. *Nature* 1985;315:758–61.
- [16] Steele MW, Breg WR. Chromosome analysis of human amniotic-fluid cells. *Lancet* 1966;i:383–5.
- [17] Yamamoto M, Wantanabe G. Epidemiology of gross chromosome anomalies at the early stage of pregnancy. *Contrib Epidemiol* 1979; 1:101–6.
- [18] Boué A, Boué J, Gropp A. Cytogenetics of pregnancy wastage. *Adv Hum Genet* 1985;14:1–57.
- [19] Roberts CJ, Lowe CR. Where have all the conceptions gone? *Lancet* 1975;i:498–9.
- [20] Caspersson T, Farber S, Foley GE, et al. Chemical differentiation along metaphase chromosomes. *Exp Cell Res* 1968;49:219–22.
- [21] Caspersson T, Zech L, Johansson C. Differential banding of alkylating fluorochromes in human chromosomes. *Exp Cell Res* 1970;60: 315–9.
- [22] Rooney DE, editor. Human cytogenetics: constitutional analysis. New York: Oxford Univ. Press; 2001. p. 99–127.
- [23] Seabright M. A rapid banding technique for human chromosomes. *Lancet* 1971;2:971–2.
- [24] Moorhead PS, Nowell PC, Mellmann WJ, et al. Chromosome preparations of leucocytes cultured from human peripheral blood. *Exp Cell Res* 1960;20:613–6.
- [25] Nowell PC. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res* 1960;20:462–6.
- [26] Yunis JJ. High resolution of human chromosomes. *Science* 1976;191: 1268–70.
- [27] Schmickel RD. Contiguous gene syndromes: a component of recognizable syndromes. *J Pediatr* 1986;109:231–41.
- [28] Langer-Safer PR, Levine M, Ward DC. Immunological method for mapping genes on *Drosophila* polytene chromosomes. *Proc Natl Acad Sci U S A* 1982;79:4381–5.
- [29] Van Prooijen-Knegt AC, Van Hoek J, Bauman JG, Van Duijn P, Wool IG, Van der Ploeg M. In situ hybridization of DNA sequences in human metaphase chromosomes visualized by an indirect fluorescent immunocytochemical procedure. *Exp Cell Res* 1982;141: 397–407.
- [30] Trask BJ. Human cytogenetics: 46 chromosomes, 46 years and counting. *Nat Rev* 2002;3:769–78.
- [31] Cheung VG, Nowak N, Jang W, et al. BAC Resource Consortium. Integration of cytogenetic landmarks into the draft sequence of the human genome. *Nature* 2001;409:953–8.
- [32] Flint J, Wilkie AO, Buckle VJ, Winter RM, Holland AJ, McDermid HE. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat Genet* 1995;9:132–40.
- [33] Knight SJL, Regan R, Nicod A, et al. Subtle chromosomal rearrangements in children with unexplained mental retardation. *Lancet* 1999;354:1676–81.
- [34] De Vries BBA, Winter R, Schinzel A, Van Ravenswaaij-Arts C. Telomeres: a diagnosis at the end of the chromosomes. *J Med Genet* 2003;40:385–98.
- [35] Solinas-Toldo S, Lampel S, Stilgenbauer S, et al. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. *Genes Chromosomes Cancer* 1997;20:399–407.
- [36] Pinkel D, Seagraves R, Sudar D. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998;20:207–11.
- [37] Albertson D, Pinkel D. Genomic microarrays in human genetic disease and cancer. *Hum Mol Genet* 2003;12:145–52.
- [38] Veltman JA, Schoenmakers EF, Eussen BH, et al. High-throughput analysis of subtelomeric chromosome rearrangements by use of array-based comparative genomic hybridization. *Am J Hum Genet* 2002; 70:1269–76.
- [39] Vissers LE, De Vries BB, Osoegawa K, et al. Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 2003;73:1261–70.
- [40] Flemming W. Zellsubstanz, Kern und Zellteilung. Leipzig: Vogel; 1882.
- [41] Painter TS. Studies in mammalian spermatogenesis: II. The spermatogenesis of man. *J Exp Zool* 1923;37:291–336.
- [42] Waldeyer W. Über Karyokinese und ihre Beziehung zu den Befruchtungsvorgängen. *Arch Mikrosk Anat* 1888;32:1.
- [43] Waldeyer W. Karyokinesis and its relation to the process of fertilization. *Q J Microsc Sci* 1890;30:159–281.