ORIGINAL RESEARCH PAPER

Volume-7 | Issue-6 | June-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# CYTOGENETICS: MODERN ASPECTS IN DIAGNOSIS OF CHROMOSOMAL ABNORMALITIES IN INDIA

**Pediatrics**

| | |
|---|---|
| **Nitu Nigam*** | Cytogenetic Unit, Department of Center for Advance Research, King George's Medical University, Lucknow-India -226003 *Corresponding Author |
| **Satyendra Kumar Singh** | Stem Cell Unit, Department of Center for Advance Research, King George's Medical University, Lucknow-India -226003 |
| **Monica Agrawal** | Department of Obstetrics & Gynaecology, King George's Medical University, Lucknow-India -226003 |
| **Amita Pandey** | Department of Obstetrics & Gynaecology, King George's Medical University, Lucknow-India -226003 |
| **Nishant Verma,** | Department of Pediatrics, King George's Medical University, Lucknow-India -226003 |
| **Neena Mohan** | Department of Anesthesia, GSVM, Kanpur-208002 |
| **Syed Rizwan Hussain** | Cytogenetic Unit, Department of Center for Advance Research, King George's Medical University, Lucknow-India -226003 |
| **Prithvi Kumar Singh** | Cytogenetic Unit, Department of Center for Advance Research, King George's Medical University, Lucknow-India -226003 |

## ABSTRACT

The correct number of human chromosomes have discovered in 1956. After that the field of cytogenetics had developed, from the initial conventional banding to the presently used molecular array Comparative Genomic Hybridisation (aCGH) techniques. Cytogenetics is crucial tool for the diagnosis of the structure and numerical changes of chromosomes. The cytogenetic test is detect most of the chromosomal abnormalities. The aim of this review to provides a brief account of the history and evolution of human cytogenetics technique in India. This article also highlights the significance of cytogenetic techniques and its role in the diagnosis of chromosomal disorders in human.

## KEYWORDS

Cytogenetics, Chromosomes, Karyotype, FISH, Comparative Genomic Hybridisation

**Introduction:**

Chromosomes are distinguish one species from one another and moreover allow the transmission of genetic information from one generation to the next generation (from parent to offspring) (1,2). In 1956, Tijo and Levan, Ford and Hamerton reported that the normally every somatic cell in humans have 23 pairs of chromosomes - making 46 in total. Half of the chromosomes come from our father, and the other half come from our mother. This includes 22 pairs are autosome and one pair of chromosomes are the sex chromosomes, X and Y and. The ova and the sperm each carry 23 chromosomes (3). In each chromosome thousands of genes present, which expressed proteins that regulate the growth, development, and metabolic reactions (4). Chromosomal abnormalities occur either missing from or extra to the normal pair, or in the arrangement of the genetic material on the chromosome such as missing, extra, switched to another chromosome, or turned upside down. An abnormalities in the chromosomes give rise to specific physical symptoms. Aneuploidy is a most common type of chromosomal abnormality due to an extra or missing chromosome.

The chromosomal pattern can be directly related to a number of genetic abnormalities. Chromosomes characterization have a considerable diagnostic importance. Cytogenetics is used for the characterization of Chromosomes. It is a photographic or computer-generated representation which arranged the chromosomes in a standard manner according to size, centromeric position, and banding and "karyotype" refers to the constitution of chromosomes of an individual (5).

**History and Evolution of cytogenetics**

Cytogenetic techniques have been developed step by step (6, 7). Eduard Strasburger in 1875 first time observe chromosomes in plant whereas Walther Flemming in 1879–1889 in animal. Flemming coined the terms mitosis, chromatin, prophase, metaphase, anaphase, and telophase (8). Cytogenetics is the study of chromosomes structure, location and properties in the cells. It comprises the chromosomal behavior in processes such as cell division during growth and development and germ cell division. Cytogenetics also includes the study of factors that cause chromosomal alterations (9).

It includes the study of chromosome number and appearance (karyotyping), the physical location of genes on chromosomes, and chromosomal behavior in processes such as cell division. Johannsen coined the term 'gene' in 1909. This triggered the beginning of modern cytogenetics. Theodar Boveri was worked on chromosome structure and function, known as the "first genetic engineer" (10). He suggested that the mitotic polarity abnormalities could be caused by an abnormal number of centromeres which recognized the asymmetrical segregation of chromosomes (10,11). Afterward, Sutton and Boveri established the "chromosome theory of inheritance". It was combined the two disciplines of cytology and genetics referred as cytogenetics. Cytogenetics means the study of chromosomes (12).

Primarily, it was challenging to determine the mammalian diploid number because the chromosomes were packed in metaphase. In the 1950s, numerous procedural enhancements, for example the addition of colchicines to arrest cells in metaphase and the use of hypotonic solution to obtain better chromosome spreads, were made (14-17). After the discovery of 46 chromosomes in a diploid cell by Joe Hin Tjio the modern human cytogenetics started in 1955. In 1956, the correct diploid number of chromosomes 46 in man was established (18, 19).

After that it was possible to describe correctly chromosome number and chromosome abnormalities. This allowed to detection of numerical chromosome aberrations such as trisomy 21 in Down syndrome (20), trisomy 18 (21), trisomy 13 (22), 45, X in Turner syndrome (23), 47, XXY in Klinefelter syndrome (24), and Philadelphia chromosome in a patient with chronic myeloid leukaemia (25). It was also reported that amniotic fluid can be used to determine the chromosome content of the foetus by culturing (26). On the basis of Denver classification (1960) (27), with revisions at the London Conference (1963) (28) and the Chicago Conference (1966) (29), metaphase chromosomes were classified into seven groups. Jau-hong Kao et al. (30) reported a chromosome classification based on the band profile similarity along the approximate medial axis.

**History and Evolution of cytogenetics in India**

In early 1960s India scientists had shown attention in human genetics and introduced cytogenetics. Prof H. Sharat Chandra in 1967 started the Institute of Genetic Studies in Bangalore. Also, Dr. G S Mutalik also started the Genetics Division at the B J Medical College, Pune in 1960s. In India, there were nearby a dozen centers such as Institute for Research in Reproduction, Cancer Research Institute (CRI), All India Institute of Medical Sciences (AIIMS) in Delhi, Grant Medical College (GMC), Sir J. J. Group of Hospitals in Mumbai, Punjab University in Chandigarh and St. Johns Medical College in Bangalore established till 1974 where cytogenetic facilities were accessible (31,32). Prof. Manorama Thomas Prof. Jai Rup Singh, and Prof. Gopinath were in the forefront in establishing human cytogenetics in India with successful German collaborations. Prof Jai Rup Singh is the founder head of the Department of Human Genetics in the Guru Nanak Dev University and also established internationally reputed Centre for Genetic Disorders. He had established the prenatal and postnatal diagnosis of genetic diseases and localized first disease causing congenital cataract gene in India. Prof. Rajiva Raman from Banaras Hindu University (BHU) started the postgraduate course on molecular and human genetics in 1999 and also instrumental in the creation of the centre of Genetic Disorders in 2008. Dr. Ishwar C Verma is a well-known pioneer who had promoted clinical and medical genetics in all over India. Prof. S. S. Agarwal established the Medical Genetics Unit at Sanjay Gandhi Post-Graduate Institute of Medical Sciences (SGPGIMS) where offers a genetics course for postgraduate medical students (32). Prof. Shubha Phadke is work on prenatal and postnatal diagnosis of genetic diseases in Sanjay Gandhi Post-Graduate Institute of Medical Sciences (SGPGIMS), Lucknow.

**Clinical cytogenetics in India**

In India, the first case of translocation Down syndrome was reported by Chaudhuri and Chaudhuri in 1966 (33). The first case of free trisomy 21 was reported in the year 1976 (34). Several studies from Western countries showed the prevalence of Down syndrome in older mothers, but studies from India showed the prevalence of Down syndrome in younger mothers under the age of 30 years (35,36). A true hermaphrodite with XX/XY mosaicism was reported (37). In 1971, Walter et al. (1971) carried out cytogenetic studies in intellectual disability and reported a case of XXXXY variant of Klinefelter syndrome (38). Manjunatha et al. (1988), first time reported Fragile-X case in India (39). Rani et al. (1974) reported the cytogenetic abnormalities in India on couples with repeated abortions (40). The sex chromosomal and autosomal aneuploidies were routinely done in India even before G banding (41-47). The prenatal diagnosis was started in 1970s (48), chorionic villi sample and amniotic fluid karyotyping from early 1980s and cordocentesis started in late 1980s (49). The details of major publications from India are highlighted elsewhere in great detail (31).

**Banding techniques**

Numerous banding techniques were developed which help in individual chromosomes identification (Figure 1). The banding techniques showed the chromosomes in details as compared to simple staining techniques with Giemsa. Caspersson et al. (1970) reported that the first type of banding using fluorescence dye quinacrine mustard or quinacrine dihydrochloride which showed a banding pattern with fluorescence microscopy (Q-banding) (50). Then, various other banding methods were recognized, such as NOR, G-, R-, and C-banding each having its own specific properties and applications (51). Later, Seabright (1971), using trypsin, was able to obtain a characteristic G-band. This gave better resolution than Q-bands as it allowed permanent preparations and avoided the use of fluorescence microscope (52). Hence, G-banding provide a way for the modern chromosomes classification (ISCN, 1985). Pardue and Gall, 1970 was first reported C-bands technique derived from centromeric or constitutive heterochromatin region, which is rich in repetitive DNA sequences and stains darkly with Giemsa (53). This stains is preferentially used C-banding technique for centromeric heterochromatin (54).

Nucleolar organising region (NOR)-banding is a technique that stains NORs of acrocentric chromosomes (55). NOR regions are situated in the acrocentric chromosomes satellite stalks and house genes for ribosomal RNA. NOR-banding technique was developed by Goodpasture et al., 1976; Goodpasture et al., 1975 which is useful in clinical practice to study certain chromosome polymorphisms, such as double satellites (56, 57).

Actually, the G-banding technique using trypsin and Giemsa became the most accepted worldwide for routine analysis. Though, the chromosome resolution of studies remained relatively limited because the total number of bands produced on metaphase chromosomes was low, and it was difficult to detect rearrangements involving small portions of chromosomes due to excessive condensation. This state was improved by the development of high-resolution banding (58), which was accomplished by coordinating the lymphocyte cultures and obtaining more cells in pro-metaphase or even prophase. High resolution banding provided better precision in the description of chromosomal breakpoints and assignment of gene loci (59).

The revolution in banding techniques made it possible for a detailed chromosomal analysis which led to the identification of several chromosomal aberrations and the discovery of the new cytogenetic syndromes (60) proximal long arm deletion of chromosome 15 in Prader-Willi and Angelman syndromes, the short arm deletion of chromosome 17 in Smith-Magenis and Miller- Dieker syndromes and Deletion 22q11.2 syndrome (Del22) DiGeorge/Velo Cardio Facial syndromes with DNA deletions with long arm of chromosome 22, could be linked to small chromosome aberrations, and the concept of the micro-deletion or contiguous gene syndrome was born (61). The G-banding became a most accepted technique for routine analysis worldwide (14, 62). The banding pattern facilitated the detection of numerous structural aberrations like deletions, inversions, translocations and duplications as well as numerical aberrations (14,63).

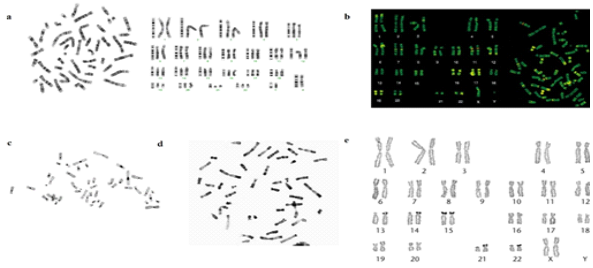

**Figure 1: Chromosome banding pattern**

(a) Giemsa banding (http ://www.monctonhigh.ca /mcGBiology/ cellular_ division.htm), (b) Q-banding (http:/ /www.mun. ca/biology/ scarr/Fig17_05a.htm), (c) C-banding (http://carolguze.com/text/442-4- chromosome_analysis.shtml), (d) R-bands(http://online library.wiley. com/doi/10.1002/0471142905.hg 0402s00/pdf), (e) NOR-banding (http://www.els.net/WileyCDA /ElsArticle/refId-a0005783.html)

**Cytogenetics Techniques**

**Fluorescent in situ Hybridisation (FISH)**

Fluorescence in situ hybridization (FISH) is a powerful molecular cytogenetic technique which which is deliberated as a new advent in the cytology field. FISH is a high sensitivity and specificity technique used for the detection of congenital disorders or chromosomal abnormalities in both the research and diagnosis (65, 66). Due to accuracy and specificity in research and diagnosis have made FISH became a pivotal cytogenetic technique (66, 67).

The detection of gene sequences presence or absence on the chromosome, is the chief concern of cytogenetic method in diagnosing of genetic anomalies (68,69). FISH is a fast and powerful technique in comparison to the conventional karyotype cytogenetic method for metaphase analysis (70,71). This technique can be enumerated and map of specific cytogenetic abnormalities, as well as a copy numbers aberrations such as, chromosomal microdeletion, translocation, and amplification. The improved diagnostic techniques have made the treatment easier and increased the life expectancy of people (65). FISH can be used to establish the order of DNA clones relative to bands, naturally occurring breakpoints, and other clones. Even more importantly, FISH permits karyotype analysis of nuclei in non-dividing cells. FISH has been used for the detection of t(2;5) (p23;q35) translocation in anaplastic large-cell lymphoma (72), for minimal residual disease in haematopoietic stem cell assays from peripheral blood stem cells of acute myeloid leukaemia (AML) patients with trisomy 8 (73), and for analysing chromosomal abnormalities of tumours in children (74). The use of FISH with the alterations of the basic method destined a revolution in detection and diagnosis of human malignancies.

**Spectral Karyotyping (SKY)/Multicolour FISH (M-FISH)**

Spectral karyotyping (SKY) or Multicolor fluorescence in situ hybridization (mFISH) are a more powerful technology, commonly used for the detections of tumor as well as clinical cytogenetics. mFISH is a multicolor karyotyping consuming whole chromosome paints as probes; this can be completed through interferometer/spectroscopy-based spectral karyotyping (SKY) or narrow-band filter-based multiplex-FISH (M-FISH) (75,76). SKY paints the whole chromosome in the similar color, while M-FISH uses several fluorescence dyes to characterise different painting probes at the same time. This offers the concurrent appearance of all 24 different human chromosomes with a single hybridisation. SKY and M-FISH have established to be enormously useful in identifying translocations and other multifaceted chromosomal aberrations. The key applications for M-FISH have been in solid tumours, which are frequently characterised by complex karyotypes, and in AML and acute lymphoblastic leukaemia (66,77).

**Array Comparative Genomic Hybridisation (aCGH)**

Microarray-based comparative genomic hybridization (array CGH) is a newly emerged molecular cytogenetic technique that was newly accepted in the clinical laboratory (78-80). Array CGH was first established as a research tool for the analysis of genomic alterations in cancer. It allows for a high-resolution evaluation of DNA copy number variations associated with chromosome abnormalities (78,79). Array-CGH is investigate the thousands and millions of genomic loci at once and it offers superior quantification of copy number and more precise evidence on the breakpoints of segments (61).

**Mutiplex Ligation Probe Amplification (MLPA)**

MLPA is a high through put technique based on relative quantification of the copy number variations in a single reaction. It is easy, faster, and cheaper method than karyotyping (81,82). It was used in clinical practice for rapidly detection of chromosomal aneuploidies (83, 84).

**Application of Cytogenetics**

**In Cancer**

Cytogenetics has become an important part of the diagnosis and management of the haematological malignancies and its effectiveness in the management of the certain solid tumors.

All malignancies shows at least one numerical or structural chromosome aberration. The contribution of chromosomal abnormalities and the deviation from the common copy number of a given chromosome (aneuploidy) in tumors have long been recognized such as, balanced chromosomal translocations can have oncogenic effects through the production of fusion proteins. In 95%chronic myelogenous leukaemia (CML) is characterized by translocation between chromosomes 9 and 22, is commonly known as the Philadelphia chromosome. Glivec is the main treatment for CML, which is a biological treatment that targets a protein made by CML cells (14,85,86). Glivec is a type of growth blocker, called a tyrosine kinase inhibitor (TKI). Tyrosine kinases are a group of proteins that cells use to signal to each other to grow. This drug stops the messages from these proteins getting to cancer cells and thus interferes with their growth. Routine conventional cytogenetics is applied to monitor the progress of this treatment. In maximum people, there is a major drop in the number of cells that carry the Philadelphia chromosome. (14,85,86). Molecular cytogenetic methods provide a snapshot of the whole molecular activities in a tumor and also help in the detection of entirely unique and unexpected functional roles of genes. These techniques allows a clinician to select the best testing approach for cancer patients.

**Chromosomal Abnormalities with syndromes**

Syndromes associated with chromosome abnormalities is shown in Table 1. Each doubted patient with chromosomal abnormalities have to go cytogenetic analysis, because karyotyping is crucial for validation of the diagnosis, determination of recurrence risk and for genetic counselling.

**Table 1: Chromosomal abnormalities associated with syndromes [3,87,88]**

| Syndrome | Chromosomal abnormality | Karyotype |
|---|---|---|
| Down's Syndrome | Trisomy 21 typeTranslocation type Mosaic type | 47, XX, + 21 46, XX,-14, +t (14;21) 46, XX/47, XX, +21 |
| Edward's syndrome | Trisomy 18 type Mosaic type | 47,XX / + 18 46,XX / 47, XX, + 18 |
| Patau's syndrome | Trisomy 13 type Translocation type Mosaic type | 47,XX /+ 13 46,XX/–14, +t(14;13) 46,XX/47,XX/ + 13 |
| Turner syndrome | Monosomy | X 45,X |
| Klinefelter syndrome | | 46,XXY or XXXY or XY/ XXY mosaic |
| Trisomy 9 | Mosaicism syndrome Trisomy | 9 47,XY,+9 |
| Cri-du-chat syndrome | Deletion | del (5 p15) |
| Wolf–Hirschhorn syndrome | Deletion | del (4p14) |
| Trisomy 8 | Trisomy 8 Mosaic | 47, XX, + 8 46, XX/ 47,XX, + 8 |
| Prader–Willi and Angelman syndromes | Deletion | del 15 (11;13) |

**In Prenatal genetic diagnosis**

Prenatal diagnosis is a variety of different techniques which can be provide health and condition of a developing fetus. In prenatal diagnosis, cytogenetic analysis of amniotic fetal cells is requires for chromosomal abnormalities (89). While, prenatal genetic diagnosis is help to determine whether a patients is at increased risk of affected fetus having genetic disorder. Amniocentesis is a well-established, safe, reliable, and accurate invasive procedure performed during pregnancy to identify chromosomal abnormalities. First time Fuchs and Riis in 1956 reported the use of amniotic fluid for the diagnosis of genetic disorder (90). Steele and Breg (1966) demonstrated that the cultured amniotic fluid cells were suitable for karyotyping (91). Cytogenetic analysis is provides the basic information for precise genetic counseling of spontaneous pregnancy losses (92). The prenatal genetic diagnosis is crucial in cases where the ultra-sonographic findings leads one to doubt on the chromosomal disorders, particularly the syndromes associated with numerous trisomies.

**Future of Human Cytogenetics**

Persistent developments in cytogenetic method have regularly exposed chromosome aberrations which are not detectable by earlier techniques. Presently, cytogeneticists are developing a stonework for molecular methods for deciphering the function, structure and evolution of chromosomes. Conventional cytogenetics using routine banding technique for chromosomal analysis are employed, remains a simple and popular method to acquire an overview of the human genome [90]. More precise detection of numerous syndromes have done by routine karyotype with M-FISH and various other molecular techniques in children. The combination of M-FISH genomic microarray (array CGH) technologies, providing a powerful approach for characterising complex karyotypes [14,93-96]. Recently, array-CGH has been widely used for the detection of copy number changes to the genome sequence from individuals with cancer and constitutional disease. Therefore, the cytogenetics techniques are crucial tools for diagnosing of chromosomal abnormalities and also help in possible treatment and management of disorder.

**References:**


1. Ciccone R, Giorda R, Gregato G, Guerrini R, Giglio S, Carrozzo R, et al. Reciprocal translocations: A trap for cytogenetists? Hum Genet 2005;117:571-82.
2. de Ravel TJ, Balikova I, Thienpont B, Hannes F, Maas N, Fryns JP, et al. Molecular karyotyping of patients with MCA/MR: The blurred boundary between normal and pathogenic variation. Cytogenet Genome Res 2006;115:225-30.
3. de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, et al. Diagnostic genome profiling in mental retardation. Am J Hum Genet 2005;77:606-16.
4. Tobias ES, Connor M, Ferguson-Smith M. Essential Medical Genetics. 6th ed. Wiley Blackwell Science; 1997. p. 57-69.
5. Williams PL. Gray's Anatomy. 38th ed. London: Churchill Livingstone; p. 193-211.
6. Gardner EJ. History of Biology, 2nd ed. Minneapolis: Burgess, 1965.
7. Hsu TC. Human and Mammalian Cytogenetics: An Historical Perspective. New York: Springer–Verlag, 1979.
8. Flemming, W., 1882. Zellsubstanz Kern und Zelltheilung. Vogel, Leipzig.
9. Hare WCD, Singh EL. Cytogenetics in Animal Reproduction. Slough: Commonwealth Agricultural Bureaux, UK. 1979.
10. Boveri, T., 1914. Zur Frage Der Entstehung Maligner Tumoren. Verlag von Gustav Fischer, Jena.
11. Harris, H., 2008. Bovert, T. [Zur Frage der Entstehung maligner Tumoren.] Concerning the origin of malignant tumors by Theodor Boveri. Translated and annotated by Henry Harris. J. Cell Sci. 121, 1–84.
12. Sutton, W.S., 1903. The chromosomes in heredity. Biol. Bull. Wood's Hole 4, 231.
13. Boveri, T., 1902. Uber mehrpolige Mitosen als Mittel zur analyse des Zellkerns. Verh. Phys-med, Ges, (Wurzburg, N.F) 35, pp. 67–90.
14. Kannan TP, Zilfalil BA. Cytogenetics: past, present and future. Malays J Med Sci. 2009;16:4-9.

15. Makino S, Nishimura E. Water pretreatment squash technic; a new and simple practical method for the chromosome study in animals. Stain Technol. 1952; 27: 17.
16. Hsu TC, Pomerat CM. Mammalian chromosome in vitro: II. A method for spreading the chromosomes of cells in tissue cultures. J Heredity. 1953; 44:23-29.
17. Ford CE, Hammerton JL. A colchicines hypotonic citrate squash sequence for mammalian chromosomes. Stain Technol., 1956; 31:247-252.
18. Tjio JH, Levan A. The chromosome number in man. Hereditas. 1956; 42:16.
19. Moorehead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res., 1960; 20:613-616.
20. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongliens. Comptes Rendus., 1959; 248:1721-1722.
21. Edwards JH, Harnden DG, Cameron AH, Crosse VM, Wolff OH. A new trisomic syndrome. Lancet. 1960;1: 787-790.
22. Patau K, Smith DW, Therman E, Inhorn SL, Wagner HP. Multiple congenital anomaly caused by an extra autosome. Lancet. 1960;1:790-793.
23. Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet. 1959; 1: 711-713.
24. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature. 1959;183:302-303.
25. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science. 1960;132:1497-1501.
26. Steele MW, Breg WR. Chromosome analysis of human amniotic-fluid cells. Lancet. 1966;1:383-385.
27. Denver Conference. The identification of individual chromosomes especially in man. Am J Hum Genet. 1960;12:384-389.
28. Hamerton JL, Klinger HP, Mutton DE, Lang EM. The London Conference on the normal human karyotype, 1963 August 2830; Cytogenetics.1963;25:264-268.
29. Chicago Conference: Standardization in Human Cytogenetics. Birth defects: Original Article Series, 11: 2, 1966. New York, The National Foundation.
30. Kao JH, Chuang JH, Wang T. Chromosome classification based on the band profile similarity along approximate medial axis. Pattern Recogn. 2008;41:77-89.
31. Madon, P.F., 2012. Conventional and molecular cytogenetics in India. In: Kumar, Dhavendra (Ed.), Genomics and Health in the DevelopingWorld. OxfordMonographs on Medical Genetics, pp. 1096–1111 (Chapter 92).
32. Dutta UR. The history of human cytogenetics in India-A review. Gene. 2016;589(2):112-7.
33. Chaudhuri, A., Chaudhuri, K.C., 1966. A survey of patients investigated cytogenetically in a medical genetics unit. Indian J. Pediatr. 33, 267–274.
34. Verma, I.C., Jacob, T.T., 1976. Down syndrome in India. Lancet 1, 1200.
35. Murthy, D.S.K., Ambani, L.M., 1978. Acrocentric chromosome association in Down syndrome patients and mothers. In: Verma, I.C. (Ed.)Medical Genetics in India 1, pp. 171–175.
36. Verma, I.C., et al., 1979. Cytogenetic profile of Down's syndrome in India. Indian J. Med. Res. 69, 147–151.
37. Udupa, K.N., et al., 1971. A true hermaphrodite with XX/XYmosaicism. Indian J.Med. Res. 59, 104–106.
38. Walter, A., et al., 1971. Cytogenetic studies in mental retardation with report of a case of XXXXY variant of Klienefelter's syndrome. Indian J. Med. Res. 59, 1072–1076.
39. Manjunatha, K.R., et al., 1988. Fragile X syndrome: the first case report from India. Genome 30, 205.
40. Rani, K., et al., 1974. Cytogenetic studies on couples with repeated abortions. Indian J. Exp. Biol. 12, 98–99.
41. Pathak, A.K., Sharma, N.I., 1968. Turners syndrome in a male child. Report of a case. Indian J. Pediatr. 35, 532–535.
42. Chaudhuri, A., 1969. 45,X-46,XX mosaicism in Turner's syndrome. Indian J. Pediatr. 36, 171–174.
43. Grover, S., Shivde, I.V., 1971. An unusual variant of Turner's syndrome. J. Postgrad. Med. 17, 195–197.
44. Phadke, M.A., et al., 1975a. Turner's syndrome—a clinical and cytogenetic study. J. Assoc. Physicians India 23, 427–431.
45. Madon, P.F., et al., 1978a. Ambiguous genitalia and the syndrome of testicular feminization. Abstracts of V Annual conference of the Indian Society of Human Genetics, Mumbai, Session B4.
46. Madon, P.F., et al., 1978b. Amalewith a uterus in an inguinal hernia. Abstracts of V Annual Conference of the Indian Society of Human Genetics, Mumbai, Session B4.
47. Verma, I.C., et al., 1978. Study of sex chromosomal aneuploidy in an Indian prison population. Indian J. Med. Res. 67, 493–498.
48. Agarwal, S.S., 1974. Antenatal diagnosis of genetic disorders. J. Assoc. Physicians India 22, 37–40.
49. Mathur, R., et al., 2002. Rapid prenatal karyotyping using fetal blood obtained by cordocentesis. Natl Med. J. India 15, 75–77.
50. Caspersson T, Zech L, Johansson C. Differentia l binding of alkylating fluorochromes in human chromosomes. Exp Cell Res. 1970;60:315-319.
51. Rooney DE, Editor. Human cytogenetics: constitutional analysis. New York: Oxford University Press; 2001.
52. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973; 273:290-293.
53. Pardue ML, Gall JG. Chromosomal localization of mouse satellite DNA. Science. 1970;168:1356-1358.
54. Sumner, A.T., et al., 1971. New technique for distinguishing between human chromosomes. Nature 232, 31–32.
55. Matsui S, Sasaki M. Differential staining of nucleolus organisers in mammalian chromosomes. Nature. 1973;246:148-150.
56. Goodpasture C, Bloom SE, Hsu TC, Arrighi FE. Human nucleolus organizers: the satellites or the stalks? Am J Hum Genet. 1976;28:559-566.
57. Goodpasture C, Bloom SE. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma. 1975; 53:37-50.
58. Yunis JJ. High resolution of human chromosomes. Science.1976;191:1268-1270.
59. Sawyer JR, Hozier JC. High resolution of mouse chromosomes: banding conservation between man and mouse. Science. 1986;232:1632-1639.
60. Garcia-Sagredo, J.M., 2008. Fifty years of cytogenetics: parallel view of the evolution of cytogenetics and genotoxicology. Biochim. Biophys. Acta 1779, 363–375.
61. Schmickel RD. Contiguous gene syndromes: a component of recognizable syndromes. J Pediatr. 1986;109:231-241.
62. Seabright M. A rapid banding technique for human chromosomes. Lancet. 1971;2:971-972.
63. Smeets DF. Historical perspective of human cytogenetics: from microscope to microarray. Clin Biochem., 2004;37:439-446.
64. Attard G, Clark J, Ambroisine L, Fisher G, Kovacs G, Flohr P, Berney D, Foster CS, Fletcher A, Gerald WL, Moller H. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene. 2008;27:253–263.
65. Ratan ZA, Zaman SB, Mehta V, Haidere MF, Runa NJ, Akter N. Application of Fluorescence In Situ Hybridization (FISH) Technique for the Detection of Genetic Aberration in Medical Science. Cureus. 2017 Jun 9;9(6):e1325. doi: 10.7759/cureus.1325.
66. Bishop R. Applications of fluorescence in situ hybridization (FISH) in detecting genetic aberrations of medical significance. Bioscience Horizons 2010;3:85–95..
67. Contexts and opportunities of e-health technology in medical care. Zaman SB, Hossain N, Ahammed S, Ahmed Z. J Med Research Innovation. 2017;1:0–4.
68. Applications of fluorescence in situ hybridization (FISH) in detecting genetic aberrations of medical significance. Bishop R. Bioscience Horizons. 2010;3:85–95.
69. Amann R, Fuchs BM. Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques. Nat Rev Microbiol. 2008;6:339–348.
70. Sujobert P, Cuccuini W, Vignon-Pennamen D, Martin-Garcia N, Albertini AF, Uzunov M, Redjoul R, Dombret H, Raffoux E. Evidence of differentiation in myeloid malignancies associated neutrophilic dermatosis: a fluorescent in situ hybridization study of 14 patients. J Invest Dermatol. 2013;133:1111–1114.
71. Garini Y, Cabib D, Buckwald RA, Ried T, Soenksen DG. Google. Patents: [May;2017 ]. 1998. Method for simultaneous detection of multiple fluorophores for in situ hybridization and multicolor chromosome painting and banding. United States patent US 5,817,462.
72. Johnson WM, Leek J, Swinbank K, Angus B, Roberts P, Markham AF, et al. The use of fluorescent in situ hybridization for detection of the t(2;5)(p23;q35) translocation in anaplastic large-cell lymphoma. Ann Oncol. 1997;8: S65-S69.
73. Dodge W, Cruz J, Zamkoff K, Hurd D, Pettenati MJ. Use of Fluorescence In Situ Hybridization to Detect Minimal Residual Disease in Hematopoietic Stem Cell Assays from Peripheral Blood Stem Cells of 2 Patients with Trisomy 8 Acute Myeloid Leukemia. Stem Cells Dev. 2004; 13(1):23-26.
74. Raimondi SC. Fluorescence in situ hybridization: Molecular probes for diagnosis of pediatric neoplastic diseases. Cancer Invest. 2000;18:135-147.
75. Ried T, Landes G, Dackowski W, Klinger K, Ward DC. Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X and Y in uncultured amniotic fluid cells. Hum Mol Genet. 1992;1:307-313.
76. Liehr T, Othman MA, Rittscher K. Multicolor Karyotyping and Fluorescence In Situ Hybridization-Banding (MCB/mBAND). Methods Mol Biol. 2017;1541:181-187.
77. Kearney L. Multiplex-FISH (M-FISH): technique, developments and applications. Cytogenet Genome Res. 2006;114:189-198.
78. Bejjani BA, Shaffer LG. Application of array-based comparative genomic hybridization to clinical diagnostics. J Mol Diagn. 2006;8:528-33.
79. Ahn JW, Coldwell M, Bint S, Mackie Ogilvie C. Array comparative genomic hybridization (array CGH) for detection of genomic copy number variants. J Vis Exp. 2015;(96):e51718.
80. Liu J, Bernier F, Lauzon J, Lowry RB, Chernos J. Application of microarray-based comparative genomic hybridization in prenatal and postnatal settings: three case reports. Genet Res Int. 2011;2011:976398.
81. Sanlaville D, Lapierre JM, Turleau C, Coquin A, Borck G, Colleaux L, et al. Molecular karyotyping in human constitutional cytogenetics. Eur J Med Genet. 2005;48: 214-231.
82. Schouten, J.P., et al., 2002. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 30, e57.
83. Carvalho B, Dória S, Ramalho C, Brandão O, Sousa M, Matias A, Barros A, Carvalho F: Aneuploidies detection in miscarriages and fetal deaths using multiplex ligation-dependent probe amplification: an alternative for speeding up results? Eur J Obstet Gynecol Reprod Biol 2010;153:151-155.
84. Gerdes T, Kirchhoff M, Lind AM, Vestergaard Larsen G, Kjaergaard S: Multiplex ligation-dependent probe amplification (MLPA) in prenatal diagnosis-experience of a large series of rapid testing for aneuploidy of chromosomes 13, 18, 21, X, and Y. Prenat Diagn 2008;28:1119-1125.
85. Hahna HP, Fletchera CDM. The role of cytogenetics and molecular genetics in soft tissue tumour diagnosis-a realistic appraisal. Curr Diagn Pathol., 2005;11:361- 370.
86. Ferrara F, Palmieri S, Leoni F. Clinically useful prognostic factors in acute myeloid leukemia, Crit Rev Oncol Hemat., 2008 Jun;66(3):18193. Epub 2007 Nov 8.
87. Mueller RF, Ian D. Young: Emery's Elements of Medical Genetics. 11th ed.London: Churchill Livingstone; 2001. p. 84-101.
88. Ponnuraj KT. Cytogenetic techniques in diagnosing genetic disorders. In: Ikehara K, editor. Advances in the Study of Genetic Disorders. Croatia InTech; 2011. p. 45-64.
89. Verma, L., MacDonald, F., Leedham, P., McConachie, M., Dhanjal, S. & Hulten, M. (1998).Rapid and simple prenatal DNA diagnosis of Down's syndrome. The Lancet 352: 9-12.
90. Fuchs, F. & Riis, P. (1956). Antenatal sex determination. Nature 177: 330.
91. Steele, M.W. & Breg, W.R. (1966). Chromosome analysis of human amniotic-fluid cells. Lancet 1: 383–385.
92. Neus Baena., Miriam Guitart., Joan Carles Ferreres., Elisabet Gabau., Manuel Corona., Francisco Mellado., Josep Egozcue & Maria Rosa Caballin. (2001). Fetal and placenta chromosome constitution in 237 pregnancies. Annales de Genetique 44: 83-88.
93. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science. 1992;258:818-821.
94. Tosi S, Giudici G, Rambaldi A, Scherer SW, Bray-Ward P, Dirscherl L, et al. Characterization of the human myeloid leukemia-derived cell line GF-D8 by multiplex fluorescence in situ hybridization, subtelomeric probes, and comparative genomic hybridization. Genes Chrom Cancer. 1999;24:213-221.
95. Speicher MR, Petersen S, Uhrig S, Jentsch I, Fauth C, Eils R, et al. Analysis of chromosomal alterations in nonsmall cell lung cancer by multiplex-FISH, comparative genomic hybridization, and multicolor bar coding. Lab Invest., 2000;80: 1031-1041.
96. Micci F, Teixeira MR, Bjerkehagen B, Heim S. Characterization of supernumerary rings and giant marker chromosomes in well-differentiated lipomatous tumors by a combination of G-banding, CGH, M-FISH, and chromosome- and locus-specific FISH. Cytogenet Genome Res. 2002;97:13-19.