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Karyotyping: Don't Banish it yet from the Field of Cytogenetics

Abhinav Dixit*, Pushpa Potaliya, Shilpi Gupta Dixit***

Abstract

Carl Wilhelm Von Nageli observed chromosomes for the first time in cells of plant in 1842. Understanding diversity and evolution of karyotypes offered the foundation for the range of variability observed in karyotypes although having origin from same macromolecule, does not guarantee the nonexistence of variation in species in number of chromosome and their detailed meticulous organization. Regardless of novel innovations in technological aspect of cytogenetics and molecular biology regarding chromosomal analysis as discussed like FISH, varieties of PCR, Microarray etc., conventional karyotyping has important role in risk stratification. Conventional banding is still considered as the gold standard. In genetic testing, it is the only one at present available for assessing the whole karyotype at once. All FISH and CGH are considered as additional powerful technique complimentary to conventional banding in cases with a futile, in complete or complex G- banded karyotype.

Keywords: Karyotyping; Cytogenetics; Molecular Biology.

What is Karyotyping?

Early in 20th century, with the progression of genetics, twirl occurred with the appreciation of chromosomes as gene carriers [1]. This was illustrated and accounted by karyotyping in which a karyotype was prepared. It was defined as the number and appearance of chromosomes in the nucleus of eukaryotic cells. Here the entire set of chromosomes in a species or an individual organism was taken into consideration. Emphasis was on substantial traits such as length, prototype of banding, site of centromere, variations in sex chromosomes etc. The chromosomes then were reorganized in a photomicrograph in a classical format in twosome, sorted in order of dimension and the site of centromere on the chromosome [2].

History of Karyotyping

Chromosomes for the first time were observed in cells of plant in 1842 by Carl Wilhelm Von Nageli [3]. This discovery was in concurrence with alterations and progressions in the field of microscopy and cellular pathology. The role of chromosomes in animal cells was exemplified and made known to the world by Walther Flemming in 1882, which is also accredited for unearthing mitosis [4]. Thereafter in 1888, German anatomist Waldeyer was the one who lay claim to name it, 'mitosis'. Latter-day preparation of karyotype was done for chromosomal analysis [5].

GrygoriiLevitsky was the pioneer worker to delineate such karyotype as the phenotypic facade of the somatic chromosomes, dissimilar to their genetic contents [2]. Further Darlington [6] and Michael JD White [7] also worked on the same concept. Yet again in 1912, ambiguity of the count of chromosome in normal diploid human cell was answered by Winiwarter[8]. In contrast to it, Painter in 1921, proposed the idea of Y chromosome and accentuated on XX and XY system with 48 chromosomes [9]. This concept was time-honored for subsequent three decades until Tjio in 1956 got his work published using cells in tissue culture. In this he used improved modus operandi by means of hypotonic solution for pretreatment, thus scattering the chromosomes. Further colchicine was used for arresting mitosis in metaphase. Chromosomes were squashed in a single plane and a certain karyotype was photomicrographed depicting 46 chromosomes [10,11].

Author's Affiliation: *Associate Professor, Department of Physiology, **Senior Resident, *** Associate Professor, Department of Anatomy, All India Institute of Medical Sciences, Basni Phase II, Jodhpur, Rajasthan-342005.

Corresponding Author: Shilpi Gupta Dixit, Associate Professor, Department of Anatomy, All India Institute of Medical Sciences, Basni Phase II, Jodhpur, Rajasthan-342005.
E-mail: shilpidr@gmail.com

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The normal human karyotype contains 22 pairs of autosomal chromosomes with one pair of sex chromosomes. In females the latter is indicated as XX whereas in males it is XY. Disparity from this customary pattern leads to innate abnormalities. Understanding diversity and evolution of karyotypes offered the foundation for the range of variability observed in karyotypes although having origin from same macromolecule, does not guarantee the nonexistence of variation in species in number of chromosome and their detailed meticulous organization. Godfrey and Masters illustrated and furnished that karyotypes have an indispensible role in elucidating noteworthy difference in diploid numbers between closely linked species [12].

Garrod and Batson in 1902 treasured the role of chromosomes and inferred that alkaptonuria is a recessive disorder. They were the earliest to put forward the idea that diseases were 'inborn error of metabolism' [13,14]. The ground for establishing molecular basis of inheritance is accredited to their research on alkaptonuria, cystinuria,pentosuria and albinism in 1923 [15,16,17]. Medical genetics and its role came much into reality with the understanding and assertion of linkage in humans in the X-linked lineage by Bell and Halden in 1937 [18]. Lately eugenics movement had fallen into disrepute. Being behind the schedule medical genetics was stepping forward in an incredibly delayed manner, thus emerging predominantly after the end of World war II in 1945. It showed brisk spurt in the subsequent half of 20th century and persisted till 21st century[19]. The world was oblivious of massive impact of double helix model of DNA in 1953 on the course of human disease [20].

Among wide range of procedures that permitted effortless enumeration of chromosomes, diverse inventions materialized in context of anomalous atypical chromosome or their count. In disorders such as Down's syndrome, type of chromosome defect as 'trisomy' was anecdoted by exploring and studying karyotypesand this fact was established that individuals bearing it had an added duplication of chromosome 21. This was explored by a French cytogeneticist Jerome *Lejeune*, with Marthe Gautier and Raymond Turpin in 1959 who identified an extra small chromosome as the cause of Down's syndrome, which was called Mongolism at that instance [21]. All these aberrations associated to non-disjunction were responsible for forming, cells with aneuploidy. Then further such numerical anomalies along with sex chromosome defects were also found, as in Turners syndrome (XO), Kleinfelters syndrome (XXY), Patau Syndrome (trisomy of 13), Edwards syndrome (trisomy of 18).

The field of Cytogenetics took a step ahead in 1960, during its course with the breakthrough of petite chromosome in WBC of Chronic Myeloid Leukemia suffering individuals. Peter Nowell and David Hungerford who found this were researchers in University of Pennsylvania and termed this abnormally small chromosome as 'Philadelphia chromosome' [22]. An American human geneticist further provided the elaborated explanation of this,Rowley demonstrated that this petite chromosome is a consequence of translocation of chromosome 9 and 22. When she published her findings, she stated that definite translocations form the basis of specific diseases, in contrast to the conventional outlook of the cause of cancer which gave modest significance to chromosomal abnormalities. Thereafter the identification of Philadelphia chromosome formed the foundation of diagnosis of cancer [23].

More sophisticated techniques like Quinacrine mustard fluorescence staining (Q-banding) were used by Caspersson in 1960s in identification and interpretation of various banding prototypes for each chromosome pair [24]. It made feasible to discriminate chromosome then on basis of parallel running horizontal bands. So, the chromosomes concerned in translocations were then effortlessly caught up. Other than that, deletions and inversions were identified and acknowledged within individual chromosome by a variety of other banding procedures as G-banding by means of trypsin and Giemsastaining that was developed in 1970's. It showed light and dark stained bands. R-banding was reversal of G-banding where dark areas were euchromatic and bright ones heterochromatic. C-banding was done to stain centromere. T-banding and silver staining was also engaged in cytogeneticsto perceive telomeres and the nucleolar organization region-associated proteins. These high-resolution bandings stained the chromosomes at some stage in prophase and early metaphase preceding the utmost condensation. At this juncture the chromosomes are extended maximally, so numbers of bands are evidently detectable. This allows the detection of less evident abnormalities that are left out by and large in conservative bandings [25].

In 1958, Ford used the fresh *bone-marrow* to examine a Klinefelter's karyotype in alliance with Lazlo G. Lajtha [26]. Medical cytogenetics had a rumble in 1959. The previous Ford method was then substituted by Moorhead by Leukocytes. So it unwrapped prospects for other medical expertise such as Pathology etc [27]. Afterwards cells extracted from amniotic fluid, cord blood, tumour and tissues (including skin, umbilical cord, chorionic villi, liver

etc.) were also cultured and scrutinized for chromosomes.

Uses in Clinical Setting

Whole understanding of a karyotype is to pin-point the associated chromosomal abnormality. It can be a numerical abnormality (Aneuploidy) as Trisomy 13,18,21,47 or monosomy X or a structural abnormality (Deletions/ Microdeletions) as in Wolf-Hirschhorn's syndrome, Cri-du-chat, Prader-Willi syndrome, Angelman syndrome, Miller-Diecker syndrome, WAGR syndrome, William's Syndrome, 22q11.2 Deletion syndrome [28,29]. Its application is also seen in translocations (such as Robertsonian, Reciprocal Translocation, acute promyelocytic leukemia, Chronic Myeloid Leukemia), isochromosomes (Xq, 12p), chromosome breakage (as Xeroderma pigmentosa, Ataxia-telangiectasia, Fanconi Anemia, Bloom syndrome, Hereditary non-polyposis colorectal cancer). Besides this, structural abnormalities such as ring chromosomes and inversions can also be identified as diagnosis of various cytogenetic disorders. For cancers, All-Aneuploidy theory is clearly suggestive that aneuploidy is responsible for causing cancers that occurs during cell division. Majority of aneuploid cells get into apoptosis but survival producesaneuploid progeny. Karyotyping has its role in various first-line prenatal, neonatal and family genetic screening for genetic disorders [30,31].

Present Status of Use-Whether Useful or Overcome by New Methods

The conventional karyotyping was indicative of various genetic disorders. Advances in technology have happened with time. Next to archetypal karyotype cytogenetics, came forward the Spectral Karyotype (SKY Technique)[32]. It is used for real-time visualization of all the pair of chromosomes in an organism in diverse colors. Assortment of fluorophores are used for labeling chromosome-specific DNA. For this, a fluorescently labeled probe for each single chromosome is made. Spectral disparity created by combinational labeling is confined and evaluated by interferometer affixed to a fluorescent microscope. Image processing software then imparts a pseudocolour to each spectrally distinct combination, allowing revelation of individually colored chromosome. Also wherever Giemsa conventional banding is not precise enough, such procedures prove their efficacy in knowing structural chromosome aberration like in carcinoma.

Later to Spectral technique, Virtual Karyotyping came into existence which comprises of short sequences of DNA from particular loci all over genome when isolated and specified to compute the DNA copy count on a genomic scale[33,34].

Near the beginning of 1980s, fluorescence in-situ hybridization (FISH) was introduced by medical researchers [35]. Multiplex FISH facilitated analysis of numerous targets and envisage co-localized signals in a solitary sample. In this, fluorescent probes were used that combine only to those elements of the chromosomes having a soaring level of complementarities to the sequence. It was used mostly to spot and localize the incidence and lack of a particular DNA sequence on chromosomes. Analysis was attained by fluorescent microscopy and in addition, technique was used for definite RNA targets in cells, circulating tumor cells and tissue specimen. FISH is an extremely proficient molecular technique for diagnosis and prediction of plentiful diseases such as Prader-willisyndrome, Angelman syndrome, 22q13 deletion syndrome, Chronic Myelogenous Leukemia, Cri-du-chat etc. In cancers, for the diagnosis, to suggest prognostic outcomes and even remission of disease, FISH can be used. It can be useful in investigative research purposes as gene mapping or the recognition of novel oncogenes or genetic aberration that have a role in different cancers. The most recentupgradation regarding it is its integration into Lab-on-a-chip micro fluidic apparatus so that it transforms further to a convenient portable diagnostic practice. It has also evolved to permit the detection of contributing causative pathogen and in deducing evolutionary association. The fundamental principle of FISH is the similar to Southern blot, where the ability of single-stranded DNA to anneal to complementary DNA is utilized [36,37]. Apart from this, when FISH is used in analogous manner along with the comparison of the hybridization potency to evoke any main disruptions in duplication of DNA sequence in genome, it is termed as Comparative Genomic Hybridization (CGH). Kallioniemi and colleagues first introduced it in 1992 in California. The objective chromosomal abnormalities where the number of chromosomes is not affected for example reciprocal translocations, inversions or ring chromosomes can be identified by means of CGH [38].

The entire Cytogenetics of contemporary era is radically transformed and primarily has the complete dependence on the Polymerase Chain Reaction. Developed by Kary Mullis in 1983, in this technique specific region of DNA is amplified, defined by a set of two primers at which DNA synthesis is instigated by a thermo stable DNA polymerase [39]. As a result million-fold of desired sequence of DNA fragment

can be obtained. This is detectable by Gel electrophoresis. It is utilized extensively in DNA cloning, phylogeny based on DNA, diagnosis of hereditary disease, genetic fingerprinting to premature diagnosis of infectious as well as malignant diseases as leukemia & lymphomas. Even chromosome definite DNA is made by use of PCR from chromosomes arranged in a dual laser flow cytometer. It is of immense importance in gene mapping and proved to be a considerable aid in scrutinizing the evolution of sex determining mechanism in vertebrates. In further advances as multiplex PCR in a specified interval, multiple target sequence can be amplified by including more than one pair of primers in the reaction. Other adaptation of it includes Nested PCR, Reverse Transcriptase PCR, Semiquantative PCR, Real-time PCR. The constraint with PCR is DNA polymerase that is prone to error; this moreover leads to mutation in PCR generated fragments. Apart from this previous information is always essential for the sequence to generate the primers [40].

Recent advances as Microarray analysis involve infringement of a cell, separating its genetic contents, recognizing all the genes that are present in that exact cell, and creating a record of those genes. Tse Wen Chang conceptualized this in 1983 in his work where the microarrays were used for the first time as antibody matrix [41]. In this two dimensional array which is on a firm substrate as glass slide or silicon thin film that arrays huge sum of genetic material is a high screening miniaturized, multiplexed and parallel processing and detection technique. DNA microarray out of all other is one of the sophisticated techniques in molecular cytogenetics nowadays. Besides this, other innovative microarrays are developed out of which the most recent is microarray of nuclear magnetic resonance micro coils, the technique of which is under currently under progression. Its major application is gene expression analysis and genetic variation analysis. It is exceptionally expensive so the current focus is to make it cost effective and more accessible within the reach of health care sector of masses [42].

Karyotyping involving banding techniques with stains is somewhat economical and is a good first line test for individual with dismorphic features, growth concerns, learning disabilities etc. One of the major limitations to it is the inability to capture miniature deletion or rearrangements. FISH is capable of figuring these small deletions, duplication or if there is any slight chromosomal rearrangement, however there has to be a doubt regarding chromosomal region or gene might be involved earlier to testing. Banding techniques has added massively to the detection and accurate understanding and elucidation of chromosome aberrations. It is emphasized by the prominent workers of the field that in medical genetics, chromosome analysis is incomplete without the banding.

The procedure is cost effective but analysis is to be done by expertise that is well trained health care professional only. The core limitation associated with it is the low resolution. The resolution of banding analysis is such that it can merely identify cryptic genomic imbalances as small as 3Mb of DNA. Moreover banding pattern are restricted to mitotically active cells with the added setback of the complexity drawn in interpreting highly rearranged chromosomes with a single toned monochrome banding pattern.

Regardless of novel innovations in technological aspect of cytogenetics and molecular biology regarding chromosomal analysis as discussed like FISH, varieties of PCR, Microarray etc., conventional karyotyping has important role in risk stratification. Conventional banding is still considered as the gold standard. In genetic testing, it is the only one at present available for assessing the whole karyotype at once. All FISH and CGH are considered as additional powerful technique complimentary to conventional banding in cases with a futile, in complete or complex G- banded karyotype.

Considering the usefulness of conventional karyotyping, a dedicated cytogenetics lab with clearly defined standard operating procedure quality control and a successful working relationship with the clinicians is needed for an increase in the success rate of conventional karyotyping.

Role in Research

The world has moved to age of nanotechnology in medical research. This simple cytogenetic procedure as karyotyping, still has a vital role to play be it stand alone or in juncture with other analysis procedures. Ability of this fundamental technology and its relevance in clinical settings even in this modern era, makes it an important diagnostic tool in medical cytogenetics.

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