Clinics in Pediatrics

Review Article
Published: 27 Jun, 2018

A Brief History of Genomic Diagnosis: From Chromosome to DNA Array

Hidefumi Tonoki*

Department of Pediatrics, Tenshi Hospital, Japan

Abstract

In Pediatric practice, chromosome analysis is often performed. This is because it is essential to diagnose chromosomal abnormalities such as Down syndrome. This test, which was developed in the second half of twentieth century, has been replaced in the 21st century by comparative genomic hybridization using DNA arrays. In the past sixty years, genome analysis techniques have progressed rapidly. Chromosome analysis technology has evolved into the G-banding method, high-resolution method, and fluorescence *in situ* hybridization since human chromosome testing methods was established in the 1950s. On the other hand, gene cloning technology was established in 1980s and many disease-causing genes had been isolated using mainly positional cloning technique. The results of the Human Genome Project provided the foundation of genome testing and exome sequencing approach aided by next-generation sequencers now are revolutionizing modern genetic practice. I would like to outline the transition of genome diagnosis over the past sixty years.

Keywords: Chromosomal abnormalities; FISH; DNA arrays; Exome Sequencing

Classic Cytogenetics

Discovery of chromosome anomalies: Anyone who is a pediatric physician would know that Down syndrome is a chromosomal anomaly. It was in 1956 that Tjio and Levan confirmed that the number of human chromosomes was 46 [1]. Three years later, a French Pediatrician, Jerome Lejeune in 1959, reported a first patient with chromosome anomaly. A disease that had been called Mongolism since London's physician John Langdon Down reported for the first time in 1866 was proved to be caused by an excess of chromosome 21 [2]. Furthermore, within a few years after the discovery of trisomy 21, chromosomal abnormalities were found in many congenital malformation syndromes; monosomy X in patients with Turner syndrome, XXY in those with Klinefelter syndrome, and partial deletions of the short arm of chromosome 5 in patients with cat cry syndrome, and so on. Chromosome observation with optical microscopes was the first practical technique for genomic analysis and became the most popular diagnostic method for congenital malformation syndromes. At about the same time, it was known that so-called prenatal diagnosis was performed; a chromosome analysis was successfully done from fetal cells in amniotic fluid.

G-banding and gene mapping: Chromosomes can only be seen during a period of cell division. When we analyze chromosomes of patients, we usually collect heparinized peripheral blood and send it to a laboratory. Blood should not be frozen nor saved for a long period. Lymphocytes isolated from peripheral blood were cultured for 72 hours at 37°C, and then were added with Colchicine to synchronize to the metaphase, middle stage of cell division, and then observed with an optic microscope. In early years, chromosomes were simply stained with Giemsa solution. 46 chromosomes were classified into groups A-G according to the size and the position of the centromere. The Down syndrome, therefore, was also called G trisomy since the smallest chromosome, chromosome 21, belongs to group G. In the early 1970s, it was found that unique stripe patterns characteristic for respective chromosomes were detected when trypsin was acted prior to Giemsa staining. This new banding technique, G-banding, had enabled us to distinguish 22 autosomes and two sex chromosomes easily under an optical microscope (Figure 1). An international conference was then held at Paris in 1971, and the designation of the bands for each chromosome was established [3]. The G-banding method had provided fine identification of chromosome regions according to the bands and allowed precise description of break points in structural abnormalities. Thereafter, many reports describing relations between clinical phenotype and structural abnormality of chromosomes such as translocation, inversion, and deletion were accumulated one after another. These reports encouraged to create a human gene map in which gene loci for genetic diseases were localized onto

OPEN ACCESS

Correspondence:
Hidefumi Tonoki, Genetics Center, Department of Pediatrics, Tenshi Hospital, N-12, E-3, Sapporo 065-8611, Japan, Tel: 81117110101; Fax: 81117511708;
E-mail: hidefumi.tonoki@tenshi.or.jp

Received Date: *23 May 2018*
Accepted Date*: 20 Jun 2018*
Published Date*: 27 Jun 2018*

Citation:
Tonoki H. A Brief History of Genomic Diagnosis: From Chromosome to DNA Array. Clin Pediatri. 2018; 1: 1002.

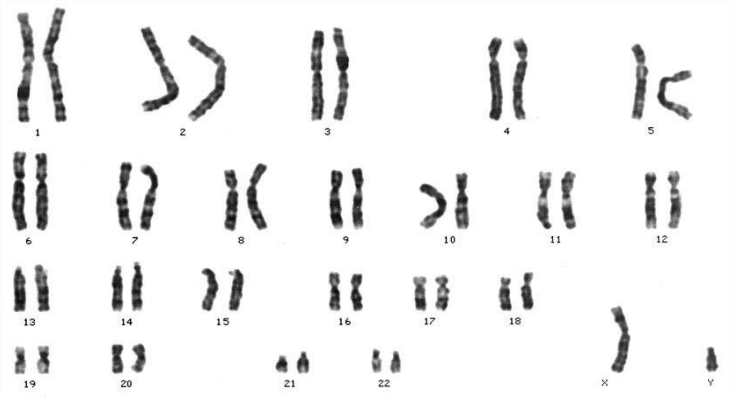

Figure 1: G-banded Chromosome showing normal male karyotype.

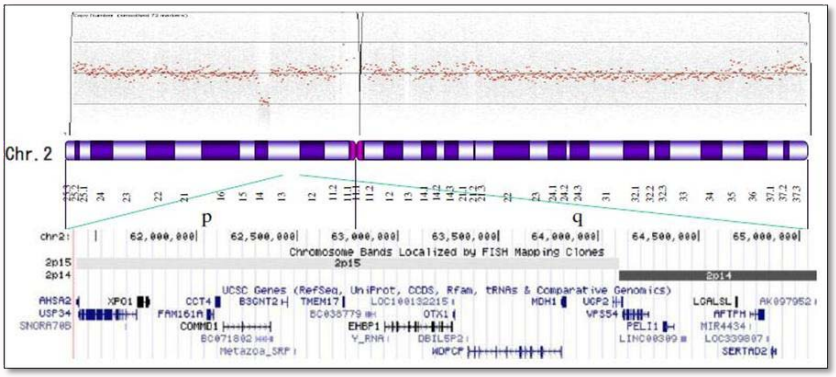

Figure 2: Array CGH result showing 3.7 Mb deletion at 2p15-p14.

bands of chromosomes.

Positional Cloning and Molecular Cytogenetics

Molecular genetics and positional cloning: The 1970s was also the earliest stage of molecular genetics. In 1972, P. Berg succeeded in the creation of recombinant DNA [4]. A few years later, T. Maniatis developed a gene isolation method (screening method of Gene library) [5]. Based on the technology of molecular cloning, an attempt to identify a causative gene of a disease started. Chromosome defect or linkage information is clue to this approach namely positional cloning. It was supposed that disease-responsible genes were located at the deleted regions or breakpoints if the patient had a novel chromosome defect such as a deletion, a translocation and an inversion. In the 1980s, high-resolution banding method was developed to analyze the chromosome in a more stretched form with finer binding pattern, which expedited gene mapping and positional cloning. It was 1987 that the causative gene for Duchenne type muscular dystrophy was identified by positional cloning method [6]. It was also in this age that Prader-Willie Syndrome was mapped to 15q11.2 and Beckwith-Wiedemann syndrome to 11p11.5.These are the results of careful analysis of minute deletions and duplications by the high-resolution banding method.

FISH molecular cytogenetics: The novel molecular cytogenetic testing method called FISH (fluorescent in situ hybridization) became applicable in the 1990s. Advanced molecular cloning technique made it possible to incorporate very large human DNA fragments into artificial vectors constructed from yeast or bacterial chromosomes. Labeled with a fluorescent dye, hybridized directly onto a slide glass with a spread of chromosomes, the DNA fragment of interest gave fluorescent signals at particular chromosomal site where the fragment originally located (in situ hybridization). By using specific DNA fragment as a probe, FISH made it possible to prove the minute deletion, and to identify the cutting point of the chromosome rearrangement such as translocation and inversion. And if one can find a minute deletion with FISH test, it means that there may be a responsible gene close to the DNA fragment used as a probe. Discovery of responsible genes for Prader-Will syndrome, Williams syndrome and Sotos syndrome were also achievements of this era, and FISH test in tern became an effective diagnostic tool for these micro deletion syndromes.

Post Genome Genetics

DNA array and genomic analysis: By 2003, the entire human genome had been completely sequenced by the Human Genome Project [7]. We now know that the human nucleus genome was made up of approximately 3.2 billion base pairs per haploid, and it included constitutional genes of approximately 25,000. And we had retained all the nucleotide sequences of all genes. The years after the achievement of Human Genome Project is called the post-genome age. The need of isolation and cloning of responsible genes that had long been the most important goal for many geneticists had been lost. Because we had already got them. Understanding a relationship between gene mutations and disease phenotype was considered as the next important issue. At first, huge efforts have been taken to find associated genes for the so-called common diseases or multi-factorial

diseases, including hypertension, psychiatric diseases, diabetes, cognitive disorders, and cancers. Finding associated genetic factors has been expected to dramatically improve the medical care for patients with these diseases. DNA arrays were newly developed tool to analyze genetic characteristics at vast number of genomic regions simultaneously from copy number variation to single nucleotide changes. There are about a million bases in the genome which are different by person namely SNP (single nucleotide polymorphism), and some of them are supposed to influence gene function. A million DNA fragments are arranged in an orderly manner on the surface of the flash memory-sized matrix, and a single base difference in the subject DNA fragment is identified at the same time. Although many associated genes for such many common lifestyle-related diseases in this way were revealed so far, most of them have not been practically helpful in the treatment of patients. This is because in most cases the risk for disease development in persons with a certain SNP was estimated to be very small. If a man had been genetically diagnosed to be at risk for a certain disease because he had a specific SNP, should he have taken any effort if he was told the risk was as small as 1.2 times compared to the general population? On the other hand, another DNA array application, so-called CGH (Comparative Genome Hybridization) enabled us to determine a copy number change in a small region that cannot be judged by chromosome analysis. Imagine an array of hundreds of thousands of pieces of DNA from whole human genome ordering from p terminal of the chromosome 1 to the end of chromosome Y waiting for making hybridization with labeled DNAs isolated from patients. The sensor and the computer to perceive the number of copies and output a precise site and length of deletions or duplication and a list of genes involved in such regions of copy number variation. Many patients with congenital anomalies who had not been genetically diagnosed with conventional methods were revealed to have genetic reasons with this new tool (Figure 2).

Exome sequencing: Further remarkable evolution in genetic testing in the post-genome age was the exome sequencing. In 2011, Sarah B. Ng University of Washington reported in Nature Genetics journal that they identified one of the causative gene for Kabuki syndrome. Gene mutations causing many popular congenital malformation syndromes had been isolated through positional cloning by the 2000s, while Kabuki syndrome was one of such diseases of which responsible genes yet to be identified. Without knowing neither biochemical characteristics nor cytogenetic aberrations in Kabuki syndrome, a novel challenging method, exome sequencing, was employed to screen exon sequences in the vast majority of genes in patients with the syndrome. With the aid of next generation sequencer, we now can determine the coding sequences of most of genes of individual simultaneously by whole exome sequencing. The first success of exome sequencing for the Kabuki syndrome encouraged many clinicians to have a chance to identify a causative genetic mutation in rare diseases. In Japan, a national research project started few years ago using array CGH and exome sequencing to screen genetic defects in order to find novel genes responsible to rare congenital disorders.

Next Generation Sequencer and Pediatric Clinic

Clinical genetics: Now, we live sixty years after the discovery of trisomy 21 as a cause of the Down syndrome. This was the first evidence that genomic defects are causes of diseases. After PCR direct sequencing method were developed in the 1990s, target gene sequencing was a powerful diagnostic means in Pediatric practice to prove clinical diagnosis of congenital errors of metabolism, connective disorders, and malformation syndromes. To have precise diagnosis of such a rare disease with target gene sequencing strategy, pediatricians must make a possible diagnosis and designate target genes to be sequenced. Exome sequencing has upset this strategy. Today when we diagnose a patient with rare disease and the patient wants to know the genetic cause, what we need to do is sending an e-mail and blood to a certain laboratory. Whole exome sequencing may detect a mutation in a gene which is unexpected or novel as a causative gene. Then, we need to think again. What is important when facing to patients with rare diseases? What guarantee the accuracy of diagnosis? Suppose that a paper concluded that MLL2 mutations were found in seven out of 15 patients with Kabuki syndrome. The denominator is the number of patients who have been judged by clinical diagnosis. What I want to say is that accuracy of this clinical diagnosis is fundamental of this issue. If it is iffy, existence of gene mutation in the patient cannot make sense. Clinicians should take pride in their clinical diagnosis and think they must be well respected. In case that the diagnosis cannot be made easily, try to record a detailed clinical phenotype and a family history before you ask genetic analysis. Before testing with array CGH, pediatricians are expected to understand the indication of this testing. Duplicated or deleted region usually involve several genes which may cause contiguous gene syndromes. Therefore, patients manifesting multi-genetic phenotype, such as undiagnosed congenital malformation syndromes with mental retardation, are most appropriate candidate for this testing, while genetic disease with autosomal recessive mode of inheritance is out of indication. Array CGH has better sensitivity for genomic imbalance detection than G-banded chromosome analysis, but it does not detect any type of balanced structural anomalies which are relatively easily found through chromosome analyses. Mosaicism of chromosome abnormality is another example that array CGH has a disadvantage in comparison to cytogenetic examinations. I would like to emphasize the importance of clinical diagnosis and classical G-banded chromosome analysis even in the age of molecular biology.

NIPT advanced method for prenatal diagnosis: Now, we obtained another strong diagnostic tool for prenatal genetic diagnosis. According to this new technology called Non invasive prenatal testing (NIPT), we are able to judge whether a fetus was suffering from chromosome anomalies such as Down syndrome without taking amniotic fluid from a pregnant woman. In the circulating blood of the mother exists a very small amount of cell free DNA derived from villi of the fetus. Using next generation sequencers, this method visualizes the genomic imbalance of the fetus. Achieving very high sensitivity and specificity, NIPT may become a standard method to screen chromosomal defect in fetuses because it is safe and reliable. On the other hand, there rose several ethical issues including, cost, commercialism which may stir up our fears of having a baby with Down syndrome, and question why only Down syndrome is a target of prenatal diagnosis, leaving many more serious diseases undiagnosed prenatally. It will have to be kept in mind to see if it will not be conducive to eugenic thought. In the summer 2017, it was reported that Down syndrome in Iceland had almost disappeared. Now it is necessary to consider whether the advancement of technology helps for the happiness of the patient, family, and the society.

References

1. Tjio JH, Levan A. The chromosome number of man. Hereditas. 1956; 42(1-2):1-6.

2. Lejune J, Turpin R, Gautier M. Chromosomic Diagnosis of mongolism. Arch Fr Pediatr.1959. 1959;16:962-3.

3. Paris Conference Standardization in Human Cytogenetics. The National Foundation – March of Dimes. Cytogenetics. 1972;11(5):317-62.

4. Jackson DA, Symons RH, Berg P. Biochemical method for inserting new genetic information into DNA of Simian Virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli..Proc Natl Acad Sci U S A.1972;69(10):2904-9.

5. Maniatis T, Hardison RC, Lacy E, Lauer J, O'Connell C, at al. The isolation of structural genes from libraries of eucaryotic DNA.Cell. 1978;15(2):687-701.

6. Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM. Complete cloning of the duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell.1987;50(3):509-17.

7. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. International human genome sequencing consortium. Initial sequencing and analysis of the human genome. Nature. 2001;409(6822):860-921.

8. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet. 2010;42(9):790-3.