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Conventional Karyotyping in Down Syndrome: Diagnostic Relevance in the Molecular Era

Rishika Sharma
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow – 227105, Uttar Pradesh, India

Janhavi Verma
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow – 227105, Uttar Pradesh, India

Somali Sanyal*
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow – 227105, Uttar Pradesh, India

Garima Awasthi**
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow – 227105, Uttar Pradesh, India

Corresponding author: gawasthi@lko.amity.edu**

Co-Corresponding author: ssanyal@lko.amity.edu*

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Abstract

Down syndrome (DS), also known as trisomy 21, is the most common chromosomal aberration, characterized by global intellectual disability and developmental defects. The syndrome results from an extra copy of chromosome 21, leading to gene dosage changes and normal development. Cytogenetically, DS has been classified into complete trisomy 21, Robertsonian translocation trisomy 21 and mosaic trisomy 21, with different mechanisms of occurrence and recurrence risks. Diagnosis is important for confirming disease, prognosis, prenatal diagnosis, recurrence risk and genetic counselling. Traditional karyotyping has been the gold standard of cytogenetic diagnosis for a long time, as it allows observation of the chromosomes and detection of both numeric and structural abnormalities. Traditional cytogenetics still has clinical value, despite modern diagnostic methods (FISH, chromosomal microarray analysis, quantitative fluorescent PCR, next-generation sequencing, and non-invasive prenatal testing) providing better efficiency, speed and genomic data. It is particularly required for the diagnosis of balanced translocations, chromosomal rearrangements and low-level mosaicism that may escape detection by molecular methods. Moreover, recent advances in artificial intelligence, digital karyotyping and cytogenomic platforms have significantly improved the efficiency of cytogenetic analysis. This review attempts to impart the cytogenetics of DS, the role of conventional karyotyping, the new molecular diagnostic methods, a comparison between the conventional and molecular approaches, technological advances and future perspectives on the integration of genomics in diagnostics while emphasizing the continued importance of classical karyotyping despite ongoing advances in molecular technology.

Keywords: Down syndrome, trisomy 21, conventional karyotyping, cytogenetics.

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1. Introduction

Trisomy 21 is also known as Down syndrome and is the most common of the human survivable chromosomal abnormalities and is one of the most common genetic causes of intellectual disability in the world (Antonarakis et al., 2004). This disorder is caused by extra chromosome 21 material which affects the normal dosages of genes and causes a change in several developmental, neurological and physiological processes. The discovery of the chromosomal cause of Down syndrome in 1959 (Ataman et al., 2012) has resulted in a great deal of scientific research that has led to an increased understanding of the genetic mechanisms, clinical presentations and diagnostic strategies associated with Down syndrome (Kazemi et al., 2016). The prevalence of Down syndrome varies by population around the world, with an estimated incidence of 1 per 700 live births, but influenced by factors such as maternal age distribution, availability of prenatal screening programs, socioeconomic status and healthcare resources (Hassold & Hunt, 2009). The most important risk factor is maternal age, since the frequency of meiotic nondisjunction increases as maternal age increases. There is a significant contribution of age-related dysfunction of meiotic spindle, irregular recombination patterns, and chromosomal instability to abnormal chromosome segregation during gametogenesis (Chiang et al., 2012). The clinical course of Down syndrome is very variable and has many organ systems affected. The clinical features are hypotonia, developmental delay, cognitive impairment, craniofacial features, congenital heart defects, endocrine abnormalities, hearing impairment, gastrointestinal malformations, immune dysfunction and hematological disorders (Kava et al., 2004). The clinical features may be more severe in different cytogenetic subtypes and in patients with different levels of chromosomal imbalance. Cytogenetically, there are three major types: complete trisomy 21, Robertsonian translocation associated trisomy 21 and mosaic trisomy 21 (Amor & Gardner, 2025). Clinically, knowing which chromosomal subtype is present is significant as each one has distinct inheritance, recurrence, and reproductive characteristics.

The conventional karyotype analysis has been a longstanding cornerstone of the diagnosis of chromosomes for decades and allows one to directly see the structure and number of chromosomes (Spinner & Ferguson-Smith, 2019). While molecular genetics has brought several new high resolution genomic techniques into the picture like fluorescence in situ hybridization (FISH), chromosomal microarray analysis (CMA), quantitative fluorescent polymerase chain reaction (QF-PCR), next-generation sequencing (NGS) and non-invasive prenatal testing (NIPT), conventional cytogenetics still holds significant diagnostic value (Rather et al., 2023). Karyotyping is a molecular technique that can detect balanced translocations, chromosomal rearrangements and mosaicism in the same test. Conventional cytogenetics is particularly useful in resource-constrained health care settings, as it is widely available and relatively inexpensive (Ozkan et al., 2023). In recent years, progressive technologies based on artificial intelligence for chromosome analysis, digital cytogenetics and integrated cytogenomic platforms have enhanced the efficiency and quality of cytogenetic diagnosis (Rosenblum et al., 2025). The cytogenetic basis of Down syndrome, molecular mechanisms in the pathogenesis of DS, clinical features, the importance of the conventional cytogenetic diagnosis, the recent development of molecular diagnostics, novel therapeutic options and future prospects in integrated genomic medicine are reviewed critically (Jaiswal et al., 2021).

2. Cytogenetic Basis of Down Syndrome

The etiology of Down syndrome is associated with anomalies on chromosome 21 and is known to have three major cytogenetic types, which have different mechanisms, patterns of inheritance and risk of recurrence (PLAIASU, 2017). Nearly 95% of Down syndrome is complete Trisomy 21, which is mostly due to meiotic nondisjunction in gamete production (Hassold & Hunt, 2009). In this case, three copies of chromosome 21 are present in all somatic cells as opposed to the

normal two copies. Most non disjunctions are in the mother's first meiotic division, but some are in the father's first meiotic division. There are several theories about the mechanism of maternal age associated nondisjunction. These include dysfunction of spindle apparatus with age, impaired chromosomal recombination, loss of chromosome cohesion and extended meiotic arrest of oocytes. The extra chromosome causes genes on the 21st chromosome to be over-expressed, which interferes with developmental pathways and with cellular homeostasis. Patients with the complete trisomy 21 usually have the typical Down syndrome phenotype with intellectual disability, craniofacial dysmorphism, hypotonia, congenital heart defects, delayed development and endocrine abnormalities. Some representative karyotypes are: 47, XX,+21; 47, XY,+21 (Antonarakis et al., 2020).

About 3-4% of cases are due to Robertsonian translocation-associated Down syndrome (Amor & Gardner, 2025). This form is a result of the long arm of the chromosome 21 fusing with another acrocentric chromosome, usually chromosome 14 or chromosome 21. Robertsonian translocation Down syndrome can be passed down through the family. In some cases, one parent could have a balanced Robertsonian translocation without any clinical symptoms as the genetic material is not lost. Meiosis, however, can be abnormal, leading to offspring with an unbalanced chromosome complement with an extra supply of chromosome 21 material. Parental chromosomal analysis is necessary for the evaluation of recurrence risk and reproductive counselling when Down syndrome translocation is hereditary. The risk for recurrence is high if either parent has a balanced translocation, especially if the mother is a balanced carrier (Pazarbasi et al., 2013). Representative karyotypes include: 46,XX,rob(14;21)(q10;q10),+21; 46,XY,rob(21;21)(q10;q10),+21.

Mosaic trisomy 21 is present in about 1-2% of Down syndrome patients and is a result of postzygotic nondisjunction during early embryonic mitotic divisions (Amor & Gardner, 2025). Some cells, then, have a normal complement of chromosomes, while others have an extra chromosome 21. The clinical features of mosaic Down syndrome vary significantly with the percentage and distribution of trisomic cells in the tissues. People with less trisomic cells may have less severe phenotypic features and relative better cognitive function. Care must be taken in the cytogenetic evaluation to make an accurate diagnosis of mosaicism since low level mosaicism can sometimes go undiagnosed if insufficient metaphases are examined. Representative karyotype: 46, XX/47, XX,+21 (Papavassiliou et al., 2009).

Table 1. Cytogenetic classification of Down syndrome

Cytogenetic Type	Frequency	Mechanism
Complete Trisomy 21	~95%	Meiotic nondisjunction
Robertsonian Translocation	~3–4%	Chromosomal translocation
Mosaic Trisomy 21	~1–2%	Postzygotic nondisjunction

Source: Adapted from Amor & Gardner (2025) and (PLAIASU, 2017).

2.1 Molecular Mechanisms of Down Syndrome

Pathophysiology of Down syndrome is not only due to an extra chromosome but also includes complex molecular and cellular defects caused by altered gene dosage, dysregulated signaling pathways, oxidative stress, and impaired neurodevelopment (Ruparelia et al., 2010).

Overexpression of genes located on chromosome 21 is primary molecular mechanism underlying Down syndrome. The extra chromosome leads to increased transcriptional activity of many dosage sensitive genes leading to abnormal cellular signaling, tissue development and multisystem dysfunction (Antonarakis et al. 2004). Gene dosage imbalances have impacts on several biological pathways, such as those involved in neuronal differentiation, response to oxidative stress, mitochondrial metabolism, immune regulation, hematopoiesis and cardiac morphogenesis (Wen et al., 2025; Perluigi et al., 2012). Many genes on chromosome 21 have strong clinical impact on Down syndrome as depicted in Table 2.

Table 2: Important Chromosome 21 Genes and Their Molecular Mechanisms in Down Syndrome

Gene	Normal Function	Mechanism in Down Syndrome	Clinical Impact
APP (Amyloid precursor protein)	Neural growth and synapse formation	Overexpression increases β-amyloid deposition in brain	Early-onset Alzheimer-like dementia
DYRK1A	Regulates neuronal proliferation and brain development	Excess kinase activity impairs neurogenesis and synaptic plasticity	Intellectual disability and cognitive dysfunction
SOD1 (Superoxide dismutase 1	Antioxidant defense against reactive oxygen species	species Increased oxidative stress due to enzymatic imbalance	Premature aging and neuronal damage
RCAN1 (Regulator of calcineurin 1)	Modulates calcineurin signaling	Dysregulation contributes to neuronal dysfunction	Neurodegeneration and cognitive decline
CBS (Cystathionine β-synthase)	Homocysteine metabolism	Altered folate and methylation pathways	Developmental abnormalities
ETS2	Transcription factor controlling apoptosis	Increased apoptosis in neural tissues	Craniofacial abnormalities and developmental delay
COL6A1	Connective tissue organization	Abnormal extracellular matrix formation	Musculoskeletal defects
IFNAR1/IFNAR2	Immune response regulation	Chronic interferon hyperactivation	Immune dysfunction and autoimmunity
RUNX1	Hematopoietic regulation	Abnormal blood cell differentiation	Increased leukemia risk
SYNJ1	Synaptic vesicle recycling	Impaired neurotransmission	Learning and memory deficits

Sources: Adapted from Antonarakis et al. (2020), Gardiner (2015), Korenberg et al. (1994), and Wiseman et al. (2015).

These include DYRK1A and APP, which are strongly associated with neurodevelopmental problems and early-onset neuronal damage (Antonarakis et al., 2020). RUNX1 is associated with the blood cancers common in people with trisomy 21. Changes of SOD1 activity may be involved in cell aging and neuron damage by oxidative stress (Korenberg et al., 1994; Antonarakis et al., 2020). In Down syndrome oxidative stress is increased because of the increased production of reactive oxygen species and decreased antioxidant defense. Mitochondrial dysfunction is also associated with neuron damage, accelerated cell aging and impaired energy metabolism. Oxidative stress is considered an important contributor to neurodegeneration, immune dysfunction, and premature aging observed in Down syndrome. The neuronal differentiation, impaired synaptogenesis,

altered neurogenesis, and dysregulated signaling pathways play a major role in cognitive dysfunction and developmental delay in Down syndrome. Neurodevelopmental abnormalities begin in fetal life and continue through postnatal development (Perluigi et al., 2012).

2.2 Conventional Karyotyping in Down Syndrome

Conventional karyotyping is one of the most important diagnostic tools in cytogenetics. In this, Giemsa stained and G-banded metaphases are viewed to determine chromosome number and structure (Spinner and Ferguson-Smith, 2019). Lymphocytes are often used for karyotyping as they can be stimulated to divide. At metaphase, the chromosomes are separated and examined with a karyogram. One of the most significant benefits associated with conventional karyotyping is the opportunity to examine all chromosomes at once. Unlike several molecular diagnostics that focus on specific regions of chromosomes, karyotyping enables the identification of numerical changes, translocations, structural abnormalities, and mosaicism in a single procedure (Shaffer et al., 2013). For complete trisomy 21, standard karyotype analyses reveal three copies of chromosome 21 in all metaphase spreads that are studied. Common examples of standard karyotypes include 47, XX,+21 and 47, XY,+21. With regard to Robertsonian translocation-related Down syndrome, karyotypes are very useful in diagnosing cases of structural chromosomal aberrations such as rob(14;21) and rob(21;21). This is particularly due to the fact that balanced translocations have significant reproductive importance (Kusre et al., 2015). Conventional cytogenetic techniques are also highly useful in diagnosing mosaic Down syndrome, whereby the ratio of normal cells to trisomic cells can be evaluated after examining different metaphase spreads. Although the pace of development in the area of molecular diagnosis is extremely fast, the importance of conventional karyotyping cannot be overlooked as a result of several benefits associated with this method. Karyotyping is cost-effective, standardized worldwide, readily available, and able to detect chromosome aberrations not be detected by molecular techniques. This type of diagnostic method is also highly significant in healthcare facilities lacking modern resources (Wilberg et al., 2022).

However, there are also some limitations of traditional karyotyping. Firstly, for traditional cytogenetic analysis to work, the presence of viable dividing cells is necessary, and this may take a long time. Secondly, traditional cytogenetics has lower resolution than molecular methods of high resolution, meaning that it cannot identify submicroscopic changes in the genome (Shen, 2023). Lastly, traditional cytogenetics needs highly skilled laboratory staff, as shown in Figure.1.

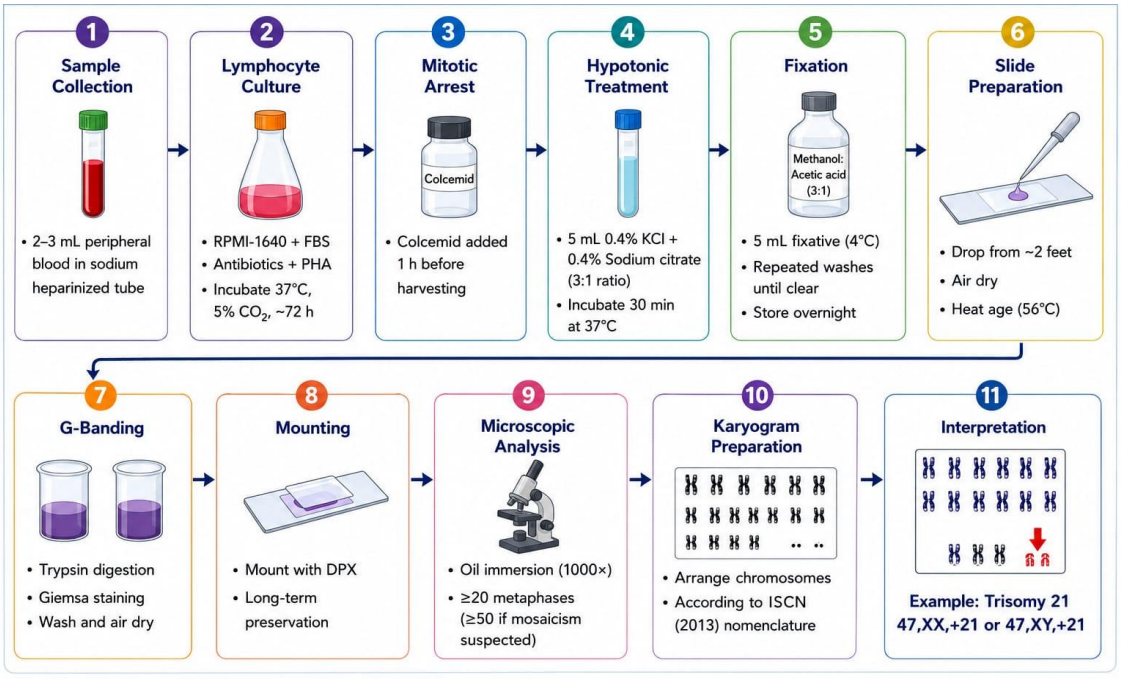

Figure 1. Workflow of the traditional karyotyping method for Down syndrome.

2.3 Limitations of Conventional Cytogenetics

Although conventional karyotyping remains indispensable, several limitations exist. Cell culture dependency increases turnaround time and may lead to occasional culture failure. The method also requires skilled cytogeneticists for accurate interpretation. Low-level mosaicism may be missed if insufficient metaphases are analyzed. Resolution is limited compared with molecular methods, preventing the detection of small deletions or duplications (Chebly, 2026; Spinner NB & Ferguson-Smith MA, 2019).

3. Molecular Diagnostic Techniques

The molecular age has brought about various high-tech genomic methodologies that have revolutionized chromosome testing and prenatal testing. Such techniques offer improved genomic resolution, better sensitivity, and faster diagnostic turnaround time (Makhamreh et al., 2025).

Fluorescence in Situ Hybridization (FISH) refers to a molecular cytogenetic method that involves using DNA probes containing fluorescence in order to detect specific regions within a chromosome (Shakoori et al., 2017). When conducting a diagnosis for Down syndrome, chromosome 21-targeting probes are hybridized to metaphase chromosomes or interphase nuclei. The FISH technique provides prompt results and can be used to conduct rapid prenatal and newborn diagnoses. It exhibits high sensitivity towards detecting chromosomal abnormalities in addition to the analysis of non-proliferating interphase nuclei. On the other hand, the use of FISH remains incomplete as only targeted regions are analyzed. This may result in unidentified chromosomal abnormalities such as balanced translocations (Namba et al., 2013).

Chromosomal Microarray Analysis (CMA) is a relatively modern high-resolution genomic approach used to detect CNVs across the entire genome (Wang et al., 2022). This method provides a highly effective means of identifying submicroscopic chromosomal abnormalities that cannot be detected using standard cytogenetic techniques. Recent research has shown that CMA has increasingly become an effective approach for prenatal testing, specifically in cases where fetuses exhibit an increase in nuchal translucency and abnormal ultrasound results (Hu et al., 2024; Xue et al., 2024). This test provides genomic analysis at a much higher resolution and is automated. However, CMA cannot detect balanced chromosomal translocations and low-level mosaicisms.

Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) represents a fast molecular testing method that is characterized by amplification of chromosome-specific markers of short tandem repeats (Shen, 2023). This test is widely utilized for fast prenatal diagnostics of common aneuploidies such as trisomy 21. Among QF-PCR's strengths, one can highlight high sensitivity, quick processing, and small amounts of genetic material required for analysis. At the same time, QF-PCR cannot be used for detecting chromosomal structural abnormalities since it only targets specific chromosomes.

Next-Generation Sequencing (NGS) allows for efficient genome-wide sequence analysis (Shen, 2023). Next-generation sequencing methods allow detecting minor variations in the patient's genetic information, including complex chromosomal abnormalities. The main advantages of using NGS involve complete genetic mapping and extremely high resolution of analysis. On the other hand, widespread use is hampered by several factors, including high costs, complex equipment, ethical issues, and bioinformatics problems.

Non-Invasive Prenatal Testing (NIPT) involves the use of cell-free fetal DNA circulating within the maternal blood to detect any chromosomal anomalies during prenatal testing (Garg et al., 2025). The NIPT technique has become highly popular due to its ability to greatly reduce the need for invasive tests such as chorionic villus sampling and amniocentesis. Recent studies have shown that NIPT is extremely sensitive and specific in detecting trisomy 21 cases (Garg et al., 2025; Wang et al., 2022). However, the NIPT method acts more like a screening technique than a confirmatory test and thus requires confirmatory genetic or molecular testing if the outcome is abnormal.

4. Comparative Analysis

The development of the molecular genomic methods has immensely improved prenatal diagnosis and chromosome analysis. However, each of the two methods exhibits unique characteristics. Standard karyotyping is still the sole approach that can effectively examine the entire set of chromosomes while identifying aneuploidy, balanced chromosome anomalies, and chromosomal mosaicism (Chebly, 2026). This is particularly important since balanced translocation involves important genetic information regarding future

recurrence risks. While molecular approaches such as FISH and QF-PCR allow quick examination, they do not conduct genome-wide analysis. In turn, although CMA significantly improves genomic analysis in terms of sensitivity to copy number alterations, it cannot detect any balanced rearrangement of the chromosomes (Yang & Jiang, 2024). The same applies to NGS, which allows highly accurate genomic identification but requires advanced technologies and significant funding. Noninvasive prenatal testing (NIPT) allowed for more efficient prenatal diagnostics. Nevertheless, it may still produce both false positives and false negatives, thus requiring follow-up cytogenetic analysis of abnormalities discovered during NIPT (Garg et al., 2025). Modern comparative studies have shown that combined molecular-genomic and traditional cytogenetic techniques represent the most successful approach for prenatal chromosomal examination (Hu et al., 2024; Xue et al., 2024).

Table 3. Comparative analysis of cytogenetic and molecular diagnostic techniques

Technique	Advantages	Limitations
Conventional Karyotyping	Detects numerical and structural abnormalities; identifies translocations and mosaicism	Requires cell culture; lower resolution
FISH	Rapid targeted diagnosis	Limited genomic coverage
CMA	High-resolution genomic analysis	Cannot detect balanced translocations
QF-PCR	Fast prenatal aneuploidy detection	Target-specific analysis
NGS	Comprehensive genomic analysis	High cost and technical complexity
NIPT	Non-invasive prenatal screening	Requires confirmatory testing

Sources: Adapted from Spinner and Ferguson-Smith (2019), Shen (2023), Zhu et al. (2025), and Lawrence (2026).

5. Clinical Relevance and Genetic Counseling:

The significance of cytogenetic diagnosis in Down syndrome goes far beyond laboratory diagnosis and directly impacts patient care, prognosis, and genetic counseling. Establishment of the type of cytogenetics is important since the recurrence risk in complete trisomy 21, Down syndrome with translocation, and mosaic trisomy 21 greatly varies. For Down syndrome with translocation, the chromosomal analysis of parents must be performed since one of the parents might carry a balanced Robertsonian translocation with a higher recurrence rate (Kusre et al., 2015). Methods of prenatal diagnosis include chorionic villus sampling and amniocentesis, which enable the examination of fetal chromosomes in at-risk pregnancies.

Postnatal diagnosis enables monitoring of development, early detection, and multidisciplinary interventions. As per report in 2024 by National Down Syndrome Society (NDSS), those who have been diagnosed with Down syndrome need lifelong screening for heart defects, thyroid dysfunction, hearing problems, blood disorders, and growth abnormalities. The earlier the interventions are carried out, the better the quality of life and prognosis of the disorder. Through genetic counseling, patients receive information about the disease pathophysiology, recurrence risk, methods of contraception, options for prenatal diagnosis, and psychological support. Therefore, cytogenetic interpretation is vital for proper patient care (Akhtar & Bokhari, 2023).

6. Advances in Recent Times

New technologies have added to the efficiency/effectiveness of cytogenetic diagnosis procedures. Digital cytogenetics, development of computerized imaging to enhance the accuracy of chromosome viewing. AI-enabled cytogenetics, identification of chromosomes is automated and has minimized interobserver variability, standardized laboratory practice. In various research studies, AI-based chromosome analysis systems have been demonstrated to enhance the diagnostic accuracy, minimize

misinterpretation and processing time (Lawrence, 2026; Zhu et al., 2025). The combination of conventional cytogenetics and genomics to develop integrated cytogenomic systems has enhanced diagnostic accuracy by providing an integrated view of chromosomal structures and genomic changes. Therefore, it can be concluded that cytogenetics has not been replaced by other medical disciplines, but rather has expanded to become a fundamental part of genomic medicine today (Federico et al., 2025).

7. Future Perspective

Future diagnostic approaches will integrate conventional cytogenetics, molecular genomics, artificial intelligence and digital imaging into one diagnostic system. The combination of these diagnostic methods can increase sensitivity, decrease diagnosis turnaround time and interpret difficult chromosomal disorders. Further improvement of the performance of automatic chromosome identification and reduction of diagnostic variability can be achieved by future work in machine learning and computational genomics. Another future priority is extension of genomic tools into under-resourced healthcare settings. However, molecular techniques are advancing rapidly, but access and cost are still major obstacles in many developing countries. The future study of genotype-phenotype relationships will contribute to the development of a personalised medicine approach for patients with Down syndrome. More research into molecular pathways may lead to the development of targeted treatments for Down Syndrome. Despite tremendous improvements in molecular diagnostic techniques, conventional karyotyping will remain relevant as it can detect balanced translocations and mosaic chromosomal disorders.

8. Conclusion

Down syndrome is still one of the most researched chromosomal disorders due to its high rate of prevalence worldwide and its associated implications. Cytogenetic diagnosis is an important procedure for confirmation of Down syndrome, prenatal screening, prognosis evaluation, treatment options and genetic counselling. Traditional karyotyping has traditionally been at the core of chromosomal analysis and continues to retain great significance in today's era of genomic medicine. The method allows for direct visualization of chromosomes and is very useful in the identification of chromosomal numerical anomalies, Robertsonian translocations, structural changes, and mosaicism. Though molecular diagnostic techniques such as FISH, chromosomal microarray testing, QF-PCR, NGS, and NIPT offer enhanced sensitivity and accuracy in addition to faster results and higher genome resolution, some of these methods have inherent drawbacks. Some molecular diagnostic methods cannot identify balanced chromosomal aberrations and chromosomal mosaicism, hence highlighting the importance of cytogenetic diagnosis. Thus, traditional karyotyping cannot be disregarded as being obsolete but rather remains an integral part of cytogenomic analysis. The progress that has been made recently in the field of AI-assisted cytogenetics, automated karyotyping, and integrated genomics platforms has contributed to the importance of cytogenetics even more. It is believed that the above-named innovations will positively affect the quality of diagnostics in the coming years.

Therefore, the combination of traditional cytogenetic methods and novel molecular approaches can be considered the best way of comprehensive examination of patients with Down syndrome. Further development of molecular techniques and genomics can contribute greatly to earlier diagnostics and genetic counseling of patients.

Author Declaration Statements

Declaration: The authors hereby declare that the manuscript submitted for consideration is an original work and has not been published or submitted elsewhere for publication. The authors take full responsibility for the integrity, accuracy, and ethical compliance of the work presented in the manuscript.

Conflict of Interest: All authors confirm that:

- Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. – Yes / Not Applicable√
- All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged. – Yes / Not Applicable√
- Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable. – Yes / Not Applicable√

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