Review Article

Recent Developments in Genetic Diagnostic Methods

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Abstract

Recent advances in genetic technology have brought in a complete change in our perceptions of genetic tests and their role and scope in clinical diagnosis. It is well known that genetic testing helps in determining the familial cause of diseases that is not limited to the present generation but also digs at the past and predicts the future. With the increasing prevalence of genetic disorders, the rapidity with which results can be obtained with the current genetic technology will be a boon to mankind. Further, the advanced techniques with rapid analytical protocols have expanded the scope for clinical discussions to decide on the treatment options for many untreatable genetic disorders. Recent diagnosis involves NGS, GWAS, aCGH, MLPA, and several PCR techniques. This review highlights the recent trends in various genetic diagnostic techniques and their scope.

Keywords: Genetic Testing; Genetic disorders; Chromosomal anomalies

Introduction

Genetic tests determine the risk of developing certain diseases in asymptomatic individuals and sometimes even the line of medical treatment in diseased subjects.[1] Genetic tests also help to determine the familial causes of diseases and provide access to the genetic information of an individual. Further, through this information, the physicians can understand whether the disease needs prevention or treatment that may be personalized. The physicians are also expected to have adequate knowledge and specific expertise in treating the diseases that have a genetic basis. This unique specialization among physicians is essential to treat such patients with confidence and to the satisfaction of patients.[2] This notion is further strengthened by the fact that our ability to generate genomic data has not equally raised our ability to interpret its significance and becomes a challenge in the clinical setting. Even though millions of people may have their genome sequenced in health care by 2025 as per the prediction of the Global Alliance for Genomics and Health, our know-how to manage them is very little. This we have to keep in mind while we are appreciating the advancements in genetic technology.

In addition, other than the clinical advantages of the information obtained from genetic testing, there are also intricate ethical, developmental, and psychological factors that require cautious consideration, especially in the pediatric section. The degree to which a child or an adolescent perceives and uses genetic information depends on their emotional and cognitive maturity. However, there are few studies that show that even 5-year-old children can theorize the biological processes including diseases and contagion.[3] The genetic test report must be relayed along with information regarding the technique used, the specificity, sensitivity, and drawbacks of the test. It would be helpful if the test reports also contain suggestions for further actions.[4]

Pre-implantation genetic testing (PGT)

Pre-implantation tests are done on embryos before they are implanted in the uterus for either pre-implantation genetic diagnosis (PGD) or pre-implantation genetic screening (PGS), whereby PGD is useful in detecting an expected phenotype and PGS is the screening of chromosomal anomalies.[5] Genetic testing of pre-implanted embryos was made by in vitro fertilization (IVF) techniques, and aneuploidies account for miscarriage in 50%-70% of couples seeking IVF treatment.[6] PGT was first performed in 1989 as an alternative for prenatal diagnosis for couples at risk of transmitting chromosomal or genetic abnormalities. This test is used as a tool for selecting in vitro fertilized embryos (from the mid-1990s).[7]

More precisely, PGD is considered where either or both the parents is/are a carrier or homozygous for a disease allele, and PGS is considered in screening the chromosomal anomalies in case of increased maternal age and other such aspects. A PGT needs a pre-implanted embryo to be biopsied. The various biopsy techniques involved include polar body biopsy, blastomere biopsy, and trophectoderm biopsy. The polar body biopsy which was first reported in the 1990s was observed with no negative effects on fertility rates and cleavage-stage development, wherein the first and the second polar body were removed simultaneously or sequentially. This biopsy is time-consuming and is pricey than blastocyst biopsy but is effective in determining the maternal errors and helpful for screening anomalies related to increased maternal age. However, this is not effective in testing post-zygotic errors.[7] Cleavage stage biopsy or a blastomere biopsy is performed in the 6-8 celled stage embryos and can contribute to maternal and paternal genetic analysis. Instead, this test was found to have negative effects on fertility rates and blastomere development.

Most PGS is done using blastocyst biopsy as this poses several advantages including less mosaicism; cheaper and better developmental prediction. The trophectoderm biopsy includes the trophectoderm cells that are extra-embryonic and allow multiple cells to be biopsied and decrease the amplification errors.[5] This procedure was previously performed using FISH (Fluorescence In situ Hybridization) but a randomized control screening using this technique reported no improvised in-vitro fertilization rates. Several genetic techniques including array comparative genomic hybridization, next-generation sequencing, and quantitative polymerase chain reaction used for pre-implantation genetic screening was proven to be modestly effective.[7] Nekkebroeck et al, 2008 and Harper et al., 2012 observed no major differences in the occurrence of inborn anomalies between the biopsied embryos and the normal (non-biopsied) embryos. Cell-free DNA was found for the first time in adult blood and has gained research interest. Assou et al., 2014 showed embryos releasing cell-free DNA where the blastocoel fluid (BF) and the culture medium (in which the embryos are grown) seemed to contain the mentioned genetic material.

This paved way for the possibility of non-invasive pre-implantation genetic testing. Although cell-free DNA was found in the BF and the culture medium, their embryonic origin has not been entirely explained. In 2013, Palini and colleagues collected 4µl of BF and 9.9pg of the median genome per sample. The investigators had achieved 95% amplificatory efficacy of TSPY1 and enabling male gender identification. Importantly this study laid the foundation for a non-invasive form of pre-implantation testing, an alternative for the biopsy (invasive) procedure in the PGT. Whereas Tobler et al., in 2013 conducted an array of comparative genomic hybridization on 96 cryopreserve embryos, they derived 63% of BF samples amplifiable after performing whole genome amplification (WGA). The results were thought to be influenced by the embryos used for the study, as those were supernumerary and were of poor clinical suitability. In the intervening time, Gianaroli et al., in 2014 derived embryonic DNA from 76.5% of studied BF (by WGA and aCGH) and reported a 97.1% concordance on ploidies of blastocoel fluids compared to trophectoderm biopsied samples.[8]

Prenatal genetic testing

Prenatal genetic testing checks the high-risk pregnancies whereby the timely information provided can help in proper pregnancy management. In 1996, the first prenatal genetic test was performed by karyotyping the cultured cells derived from the amniotic fluid. Several limitations of the karyotyping technique include invasive procedures to obtain samples, skilled analysis, time consumption, and limited resolution8. A karyotype has 99% detection rates of aneuploidies; one advantage of FISH and chromosomal microarrays over karyotype is the turn-out time. Karyotype techniques involve 7-14 days processing whereas the FISH technique takes 3-5 days, however, the FISH ultimately needs confirmation by karyotyping or chromosomal microarrays9. Different techniques including FISH, quantitative fluorescence polymerase chain reaction (QF-PCR), and multiplex ligation-dependent probe amplification (MLPA) were developed to minimize the problems faced in the conventional karyotyping procedure.[8]

These advanced techniques offer interrogations of specific gene loci, this being an advantage or a disadvantage depends on the investigated condition. A rapid genome-wide screening strategy for copy number detection was developed; the array of comparative genome hybridization (aCGH) paved the way for the detection copy variant imbalances. Modern array systems help in interrogating specific loci, whole genome, and allele-specific loci. Chromosomal Microarrays are reported to provide high diagnostic yield, and correspondingly they are found to increase the detection rate of Copy number variants (CNVs) by 6%-8% when compared with the conventional karyotyping technique.[10]

The advantages of this microarray technique are: the effective detection of several microdeletions and microduplications (even in the absence of an abnormal ultrasound), culturing of the direct amniotic samples, and avoiding divisional errors. The disadvantages of chromosomal microarray

techniques include their inability to detect conditions related to single-gene mutations, limited mosaicism detection, and their insufficiency in detecting balanced chromosomal anomalies (translocations and inversions).[11] In prenatal screening, the Whole-exome sequencing is useful in identifying de novo SNVs (Single Nucleotide Variations), indels, deletions, or duplications as observed by Carss and colleagues in 2014. Targeted counting and SNP-based methods are the two common methods used in NGS, several studies validated and reported positive: clinical validation, specificity, and sensitivity of NGS.[8]

The Cell-free fetal DNA improved non-invasive fetal aneuploidy detection by NGS (Next Generation Sequencing). Whole-genome sequencing and targeted sequencing allow detection of 13, 18, 21, X, and Y chromosome aneuploidies. The cell-free DNA (cfDNA) screening is considered as it involves a drawing of blood after 9 weeks of gestation. Even though the cfDNA tests are highly specific for aneuploidy detection, the positive cfDNA test alone is not as reliable as the origin of the cell-free DNA is trophoblastic11. Identification of genetic disorders in the fetal stage can lead to important pregnancy decisions and management. The methods and techniques for better prenatal genetic diagnosis continue to evolve and thus the opportunity for a better diagnosis is expanding. Each technique has its advantage and disadvantage, specificity for the detection of a condition, and hence, identifying the most favourable genetic test to be performed, depends on the condition is to be screened.

Neonatal genetic screening

Neonatal genetic screening helps in the screening of disorders (with the possibility of getting treated) earlier to avail of treatment. This screening must be further confirmed by more definite confirmatory tests. The first neonatal screening is done for phenylketonuria (PKU), a rare hereditary disorder that would cause a severe form of mental disorder if not treated earlier. The screening of PKU done so was simple as it involved the processing of assay of phenylalanine in dried heel-pricked blood.[12] The selection of disorders for neonatal screening plays a vital role as it defines what to be diagnosed and what not. According to the summary published by the American College of Medical Genetics and Genomics (ACMG) recommended a uniform panel for the newborn screening process. This summarizes primary disorders (must be included in the screening), secondary or additional disorders that can also be screened and disorders that have not opted for neonatal screening.

The Recommended Uniform Screening Panel (RUSP) is known to include 35, 26 primary and secondary disorders, respectively. Special considerations are to be included in the neonatal screening procedure in the screening of pre-term and sick infants, as the period in which the sample is collected determines the reliability of the test. More likely, the screening of pre-term and \sick infants report a false-positive result and this is related to certain prenatal conditions and clinical interventions.[13] The blood sample collected for the screening is analyzed through mass spectroscopy for the identification of genetic disorders associated with greater alterations in blood biochemical levels. Wherein, other conditions such as cystic fibrosis, immunodeficiency, and hemoglobinopathies can be screened by other tests.

Several genetic tests used in the newborn screening include the next-generation sequencing, PCR (Polymerase Chain Reaction), sequencing of individual genes, gene panel testing, and Genome-wide studies.[14] Many PCR techniques can be used for the screening of several disorders; scientists investigated the capability of PCR in the screening purpose. Vidal-Folch et al., in 2011 investigated the effect of ddPCR (droplet digital-PCR) in screening spinal muscular atrophy, a muscular degenerative disorder. In this study whereby the SMN1 deletion and SMN2 CNVs are detected, suggesting that the ddPCR is efficiently susceptible and be capable of newborn screening. Hao and colleagues analyzed the efficiency of real-time PCR in the screening of deafness15. Another study in the Indian population also determined the possibility of GJB2 mutations for 19.4% of non-symptomatic hearing loss (NSHL) by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Limitations of this screening include the lack of specific markers for measuring, no optimized treatment available, and the inability to screen several disorders (mitochondrial disorders, congenital lactic acidosis). The ethical considerations involved include: 1) Opting the testing process 2) Distinctness of the screening is uncertain 3) The consent to store the dried blood spots 4) Discussion of the results with other family members. Although the screening of newborn infants for several life-threatening disorders is important for the betterment of them, this involves several limitations that should also be considered.

Carrier testing

Carrier testing is performed to identify mutations in individuals considering pregnancy or women who are pregnant, in order to prevent genetic diseases in the succeeding generations. Approximately 15% of over 7000 diseases that are believed to show Mendelian inheritance are recessive in nature16. For such autosomal recessive conditions, the individuals who carry the altered gene but do not present any symptoms are called carriers. However, the offspring

of carrier couples are susceptible to a 25% possibility of exhibiting the condition. This screening method helps these carrier couples to decide on their future reproductive plans and reassure those with a negative result.

For carrier women early into pregnancy, this testing method helps them to decide on the prenatal diagnosis after counselling sessions with a genetic counsellor.[16] Apart from mainly focusing on recessive Mendelian disorders, it also focuses on other Mendelian disorders, X-linked disorders, chromosomal abnormalities, and mitochondrial diseases.[17] Carrier testing that is traditionally performed is limited to certain conditions and ethnic groups are at risk for these conditions. Recently, the carrier testing method has been changed to Expanded Carrier Screening (ECS) and is alternatively referred to as universal carrier screening or pan-ethnic screening. Expanded Carrier Screening is performed to test for many genetic conditions concurrently, and for all ethnic groups. This test can screen for over 200 conditions which have vast variations in their prevalence, treatment availability, nature of the condition, effects of the previous diagnosis, and sensitivity of the screening.[18]

A study that was conducted in 2018 has concluded that the 176- disease Expanded Carrier Screening (ECS) panel, conducted by assuming the reproductive outcomes that were observed previously among the high-risk couples is profitable to perform compared to the conventional carrier testing method.[19] According to a study conducted in 2017, ECS is offered by 16 companies of which 13 were commercial companies. Pan-ethnic screening is not a stand-alone test but a part of a network of genetic tests performed together and hence becomes difficult for the individuals to differentiate ECS from other genetic tests.[20] Thus stressing the importance of carrier testing to the patient becomes strenuous. Apart from this, there are still certain drawbacks associated with carrier testing that need to be worked on.

Presymptomatic and predictive testing

Presymptomatic and predictive genetic testing (PST) is conducted to gauge the possibility of identifying mutations that cause genetic or hereditary conditions. This testing is offered for many genetic and hereditary disorders such as neurodegenerative genetic disorders, heritable cancer syndromes, and cardiac conditions. Presymptomatic is when the positive result indicates in advance the occurrence of a disorder and predictive is when the result shows the risk of an individual to a disorder i.e. the disorder may or may not occur.[21] The Presymptomatic genetic testing will educate the individuals on their health and help

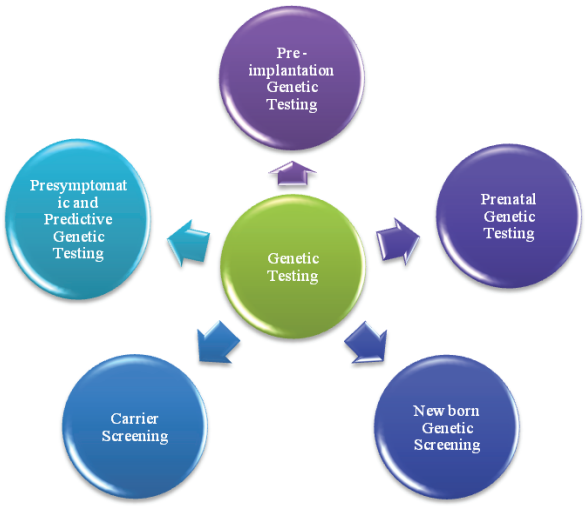

Figure 1: Types of Genetic Testing

them make informed decisions about their future treatments. PST should be conducted at the appropriate age to prevent a negative impact on the psyche of an individual. Hence PST is not recommended for people below 18 years of age for testing Adult-onset disorders unless it has a major impact on their lives.[21] This testing in minors (those who have not reached 18 years of age) may also affect patient confidentiality when the results are relayed to the parents and it also affects the person's right to not know.[22] Presymptomatic testing is helpful in many aspects, however, for minors, PST becomes a conflict of interest. Solving some of the issues associated with Presymptomatic testing in adolescents will make PST a powerful tool in diagnosis (Fig 1).

Diagnostic tools in genetic testing

To perform genetic tests, certain diagnostic tools are used, from the most conventional karyotyping to the latest Next Generation Sequencing. Karyotyping is the most basic test which is used to categorize and arrange chromosomes based on their shape, size, and banding pattern. This testing method helps to identify chromosomal ploidies and structural abnormalities such as deletion, translocation and inversion.[23] It is, however, labor-intensive and cannot identify microdeletions and other similar mutations. FISH is a more advanced technology compared to karyotyping. In this method, the DNA or in some cases the entire chromosomes are tagged with a fluorescence probe that paints the chromosomes and facilitates the analysis of these chromosomes through fluorescence microscopy or any imaging system.[24]

Another diagnostic tool used is the comparative genome hybridization (CGH). This method is used to identify CNVs (Copy Number Variants) from the sample by comparing the sample genome with a standard reference genome without culturing the

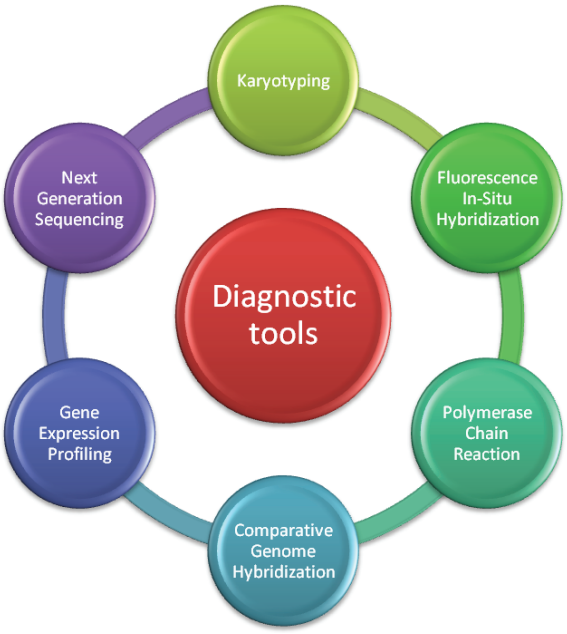

Figure 2: Diagnostic Tools used in Genetic Testing

samples.[25] The more recent method being used is the Next Generation Sequencing (NGS). This term collectively refers to the new DNA sequencing techniques that have revolutionized genomic research. This method has gained fame because of its ability to sequence the entire human genome within a day.[26] The genetic testing tools are not limited to these techniques and more advanced new techniques are being developed (Fig 2).

Conclusion

Genetic testing is a fairly new diagnostic method that has been brought into practice. This method of diagnosis has improved the quality of healthcare provision and enabled quicker intervention of professionals. Genetic testing has also made it possible to detect diseases that were previously difficult to detect and have also been useful in identifying many new disorders. Even though genetic testing has developed a lot in the past years, there are still certain setbacks regarding the result delivering, sensitivity, and quality of the tests that can be improved further to make genetic testing the ideal testing method for not just genetic and inherited disorders but also other disease conditions.

Conflicts of interest

All the authors declare that they have no conflict of interest

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References

1. Constantin, C.M., Faucett, A. and Lubin, I.M. A Primer on Genetic Testing. The Journal of Midwifery & Women s Health. 2005; 50: 197-204.
2. Lim Q, McGill BC, Quinn VF, et al. Parents' attitudes toward genetic testing of children for health conditions: A systematic review. Clin Genet. 2017; 92(6):569–578.
3. Hawkins AK, Ho A. Genetic counseling and the ethical issues around direct to consumer genetic testing. J Genet Couns. 2012; 21(3):367–373.
4. Mulcahy, G.M., Goggins, M., Willis, D., et al. Pathology and genetic testing. Cancer. 1997; 80: 636-648.
5. Sullivan-Pyke C, Dokras A. Preimplantation Genetic Screening and Preimplantation Genetic Diagnosis. ObstetGynecol Clin North Am. 2018;45(1):113–125.
6. Treff NR, Zimmerman RS. Advances in Preimplantation Genetic Testing for Monogenic Disease and Aneuploidy. Annu Rev Genomics Hum Genet. 2017; 18:189–200.
7. Harper, J. C. Preimplantation genetic screening. Journal of Medical Screening. 2018; 25(1), 1–5.
8. C. Farra, F. Choucair, and J. Awwad. Non-invasive pre-implantation genetic testing of human embryos: an emerging concept Human Reproduction. 2018; 33(12): 2162–2167.
9. Berisha, SZ, Shetty, S, Prior, TW, Mitchell, AL. Cytogenetic and molecular diagnostic testing associated with prenatal and postnatal birth defects. Birth Defects Research. 2020; 112: 293–306.
10. Wojcik, M.H., Reimers, R., Poorvu, T. et al. Genetic diagnosis in the fetus. J Perinatol. (2020).
11. Angie C. Jelin, Katelynn G. Sagaser, Louise Wilkins-Haug, Prenatal Genetic Testing Options. Pediatric Clinics of North America.2019; 66(2).
12. Holtzman, N.A. Neonatal Screening. In eLS, (Ed.). 2006.
13. Noelle Andrea V. Fabie,Kara B. Pappas,Gerald L. Feldman. Pediatr Clin N Am 66 (2019) 369–386.
14. Friedman, J.M., Cornel, M.C., Goldenberg, A.J. et al. Genomic newborn screening: public health policy considerations and recommendations. BMC Med Genomics 10, 9 2017.
15. Hao Z, Fu D, Ming Y, et al. Large scale newborn deafness genetic screening of 142,417 neonates in Wuhan, China. PLoS One. 2018;13(4):e0195740.
16. Bajaj K, Gross SJ. Carrier Screening: Past, Present, and Future. J Clin Med. 2014; 3(3):1033-42.
17. Gil-Arribas E, Herrer R, Serna J. Pros and cons of implementing a carrier genetic test in infertility practice. CurrOpinObstet Gynecol. 2016;28(3):172–177.

18. Stevens B, Krstic N, Jones M, Murphy L, Hoskovec J. Finding Middle Ground in Constructing a Clinically Useful Expanded Carrier Screening Panel. Obstet Gynecol. 2017; 130(2):279–284.

19. Beauchamp KA, Johansen Taber KA, Muzzey D. Clinical impact and cost-effectiveness of a 176-condition expanded carrier screen. Genet Med. 2019;21(9):1948–1957.

20. Kraft, S.A., Duenas, D., Wilfond, B.S. et al. The evolving landscape of expanded carrier screening: challenges and opportunities. Genet Med.2019; 21: 790–797.

21. Godino L, Turchetti D, Jackson L, Hennessy C, Skirton H. Impact of presymptomatic genetic testing on young adults: a systematic review. Eur J Hum Genet. 2016; 24(4):496-503.

22. Borry P, Stultiens L, Nys H, Cassiman JJ, Dierickx K. Presymptomatic and predictive genetic testing in minors: a systematic review of guidelines and position papers. Clin Genet. 2006; 70(5):374–381.

23. Shemilt L, Verbanis E, Schwenke J, Estandarte AK, Xiong G, Harder R, Parmar N, Yusuf M, Zhang F, Robinson IK. Karyotyping human chromosomes by optical and X-ray ptychography methods. Biophys J. 2015; 108(3):706-13.

24. Cui C, Shu W, Li P. Fluorescence In situ Hybridization: Cell-Based Genetic Diagnostic and Research Applications. Front Cell Dev Biol. 2016; 4:89.

25. Cheung SW, Bi W. Novel applications of array comparative genomic hybridization in molecular diagnostics. Expert Rev MolDiagn. 2018; 18(6):531–542.

26. Behjati S, Tarpey PS. What is next generation sequencing? Arch Dis Child EducPract Ed. 2013; 98(6):236-8.

27. Mishra S, Pandey H, Srivastava P, Mandal K, Phadke SR. Connexin 26 (GJB2) Mutations Associated with Non-Syndromic Hearing Loss (NSHL). Indian J Pediatr. 2018; 85(12):1061–1066.