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Modern Medical Genetics and Genomics

Edited by Israel Gomy

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Meet the editor

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Contents

Preface

The field of medical genetics and genomics is undergoing a constant revolution based on new breakthroughs that bring increasing insights into the etiology of rare and common diseases, which help improve the health care of individuals with these disorders.

The development of high-throughput technologies such as next-generation sequencing (NGS) has enabled the detection of inherited susceptibilities and actionable mutational profiles of tumors for tailored therapies and management. Additionally, the generation of such massive amounts of data has prompted advances in the field of bioinformatics, with regard to complex tools and numerous databases to guide the interpretation of such big data, and to deal with noninformative results such as the notorious variants of uncertain significance. Besides these technical challenges, important ethical issues must also be considered: for example, the disclosure of results from prenatal and newborn screening through NGS, and findings from population-level screening of asymptomatic individuals. Addressing such challenges is not easy. We propose that investigators must provide tentative answers for one vital question: What is the analytic validity, clinical validity, and clinical utility of genetic testing?

This book aims to depict some of the myriad applications of genetics and genomics in the practice of medicine. We present the material in three sections: (1) an introductory landscape of medical genetics and genomics and portraits of personalized genomic medicine; (2) the field of reproductive genetics and prenatal screening: the first two chapters of this section deal with the most critical factors involved in the success of embryo implantation through assisted reproductive technology, including endometrial receptivity and rates of embryonic aneuploidy; the third chapter evaluates the accuracy of invasive and noninvasive prenatal screening for predicting the risk of fetal malformations; and (3) the area of hereditary cancer genetics, wherein is presented an updated review of the clinical utility of genetic testing, as exemplified by the description of hereditary breast and ovarian cancers.

Even though the information presented here offers a very limited look at the modern-day developments and breakthroughs of the centenarian field of medical genetics, this book nevertheless provides an important glimpse of the fascinating and challenging era of personalized or precision medicine.

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Section 1

Introduction

Modern Medical Genetics and Genomics in the Era of Personalized/Precision Medicine

Israel Gomy

1. Introduction

Modern medical genetics as a well-defined field of medicine has developed so fast since its origins half a century ago that we cannot bear in mind how long time ago its roots and beginnings came from.

It can be argued that genetics overall was based on measuring the problems of human hereditary features and inherited diseases, since before the twentieth-century acceptance of Mendelian laws of heredity. Thus, medical genetics, viewed from its broadest perspective, is perhaps the oldest area of genetics and not a recent area that it is sometimes believed.

2. Modern genomics in medicine

During the years, with developments in cellular and molecular biology, the field of medical genetics expanded from a small clinical subspecialty focused at describing a few rare hereditary disorders to a recognized medical specialty whose principles and approaches are essential parts of the diagnosis and management of many disorders, both common and rare. These genetic concepts and approaches are not restricted to any one medical specialty or subspecialty, as they permeate many, and perhaps all areas of medicine.

The medical geneticist is usually a physician who works as part of a team of clinical providers, including many other physicians, nurses, and genetic counselors, to evaluate patients and their relatives for possible hereditary diseases. They characterize the patient (or proband) through analyses of personal and family history and physical examination; assess risk and possible modes of inheritance; indicate diagnostic testing; manage prevention, treatment, and surveillance; and participate in communicating to other family members at risk for the disorder.

During the twentieth century, it gradually became clear that hereditary factors were implicated in many conditions and that different genetic mechanisms were involved. Virtually, any disease is the result of the interaction of genes and environment, but the relative influence of the genetic component may be large or small. Traditionally, genetic conditions have been classified into three categories: monogenic, chromosomal, and multifactorial disorders. However, it is becoming increasingly evident that the interplay of different genes (polygenic inheritance) is essential in disease and that an additional category—acquired somatic genetic disease—should also be included.

Improvements in all areas of medicine, mainly public health and therapeutics, resulted in modifying patterns of diseases, with improving recognition of the role

of genetic factors for most of common disorders or even for the susceptibility to infectious diseases. For complex chronic degenerative diseases of adult onset, their overall contribution of heritability has been identified, as life expectancy increases and high-throughput technologies improve. These provide the opportunity for understanding the interactions between the genetic and environmental factors of diseases such as cancer, Alzheimer's disease, diabetes mellitus, macular degeneration, and cardiomyopathy.

In recent years, we have been facing the applications of modern genomics to the practice of medicine. With robust molecular biology technologies, one can identify the actionable mutations present in a tumor and establish the profile of its pattern of RNA expression, which are currently being used for determining prognosis and choosing appropriate targeted therapies for individual cancer patients. Another application is how modern genomic approaches are increasing our abilities in risk assessment and helping provide more accurate genetic counseling to patients and families affected with hereditary diseases as well as advances in prenatal diagnosis.

There are further examples of applications of genomics to individualized health care: screening asymptomatic individuals for genetic predisposition to various diseases in order to improve health care, population-based newborn screening for preventable and treatable genetic diseases, identifying couples that are carriers for autosomal recessive or X-linked diseases that could affect their children before conception, prenatal screening of the fetus for aneuploidy by maternal cell-free DNA, and applications of pharmacogenomics on the detection of individual variation affecting drug therapy, which can be used to improve therapeutic efficacy and reduce adverse events.

At the beginning of the twenty-first century, the Human Genome Project (HGP) provided a virtually complete sequence of human genome from which now derives the efforts to catalog all human genes, understand their structure and regulation, determine the extent of their variation in different populations, and uncover how genetic variation contributes to susceptibility. The whole genome of any individual can now be sequenced rather than sequencing one gene at a time. These achievements are making possible the practice of genomic medicine, which aims at applying a wide analysis of the human genome and its products, including the epigenetic regulation of gene expression, gene variation, and their interactions with the environment, to medical care.

The HGP has now been succeeded by the Human Variome Project, which seeks to compile and share the huge variation in human DNA sequence worldwide. This is potentially possible since whole exome sequencing (WES) and whole genome sequencing (WGS) have been increasingly performed in several population studies. For example, the 100,000 Genomes Project in the United Kingdom have recently achieved its goal of 100,000 genomes sequenced [1].

The study of genetics and its role in causing human disease is now widely acknowledged as being among the most exciting and influential areas of medical research. Certainly, their valuable discoveries have benefited patients and families dramatically, but this achievement will be measured by translating them into both treatment and prevention of disease.

3. Diagnostic advances

Advances over the past few years in mutation identification have provided many improvements in risk assessment, carrier detection, and prenatal diagnosis, allowing the detection of particular mutations with almost 100% accuracy. Laboratory testing for pathogenic (disease-causing) mutations is available for more than 4,500 genes associated to over 11,000 genetic conditions [2]. The better knowledge of the genes involved in hereditary disease and the rapidly

cheaper cost of DNA sequencing have permitted the identification of mutations in a patient or family member, and, therefore, the molecular diagnosis has become the standard of care for many conditions. DNA samples for testing are available not only from readily accessible tissues, such as a buccal scraping or blood sample, but also from tissues obtained by more invasive testing, such as chorionic villus sampling or amniocentesis.

For many hereditary disorders (including retinal degeneration, deafness, hereditary breast and ovarian cancer, Lynch syndrome, congenital myopathy, mitochondrial disorders, and hypertrophic or dilated cardiomyopathies), there is a substantial locus heterogeneity, that is, numerous genes are known to be mutated in different families with these disorders. When a patient with one of these highly heterogeneous disorders seeks for testing, recent advances in DNA sequencing make it possible to analyze large panels of dozens to more than 100 genes simultaneously and cost-effectively for mutations in every gene in which mutations have been seen previously to cause the disorder.

In those conditions for which even a large panel of relevant genes cannot be formulated for a particular phenotypically defined disorder, or for those genetically heterogeneous entities (e.g. intellectual disability, autism spectrum disorder) genetic diagnosis still can be achieved by analyzing all the coding exons of every gene (by WES) or by sequencing the entire genome (by WGS) to identify pathogenic mutations.

The use of large gene panels and, even more so, WGS or WES raises special issues for sequence interpretation and risk assessment. As there are more genes being tested, the number of sequence differences between a patient's DNA and that of an arbitrary reference sequence also increases; consequently, many previously undescribed variants will be found whose pathogenicity is unknown. These are so-called variants of uncertain significance (VUSs). This is the case, for example, of a missense mutation that results in the substitution of one amino acid for another in the encoded protein.

Thus, the interpretation of variants is a challenging and demanding area for all professional geneticists who provide molecular diagnosis. The American College of Medical Genetics and Genomics has recommended that variants be categorized into one of five classes: pathogenic, likely pathogenic, of uncertain significance, likely benign, and benign variants [3] Specialists in molecular diagnostics, human genomics, and bioinformatics have developed a series of criteria for addressing the mutation status. In most cases, none of these criteria is absolutely definitive but must be considered together to provide an overall assessment of the pathogenicity of a variant. Only those variants with a high probability of being pathogenic are communicated to the health professional and patient. It is arguable whether the testing laboratory should disclosure all VUSs, at the same time remaining available for updating as new information allow reclassification as either benign or pathogenic.

Despite all the time and effort put into interpretation, it is still impossible to ensure any clinical significance to the vast majority of all variants found through next-generation sequencing (NGS). There is a general concern that individuals and their clinical providers, when confronted with VUSs, will require additional unnecessary testing, with the potential for finding results with even more VUSs, thereby increasing patient's uncertainty and anxiety. Moreover, there is the additional concern that even when a variant is known to be pathogenic and highly penetrant in families with multiple affected individuals, the true penetrance of a variant when it is found in individuals with a negative family history may be much lower.

Therefore, risk assessment and genetic counseling in this context are challenging processes and depend on continually addressing recently available information and communicating it properly to healthcare providers and patients.

4. Ethical dilemmas

Each new advance in genetic technology has simultaneously brought new ethical concerns and raised new dilemmas about how science will be applied and utilized in medicine. At the center of this is the recognition that an individual's genetic background is fundamental to both their identity and possible disease susceptibility. The most controversial field is prenatal genetics and reproductive choice, though national legal frameworks and cultural practices vary widely worldwide. The debate surrounding the early ability to perform prenatal diagnosis for Down syndrome through invasive procedures in the mid-1960s is compared to the new technology that makes it possible to perform detailed genetic screening of the fetus on cell-free fetal DNA in the maternal blood or on embryos created through in vitro fertilization for preimplantation genetic diagnosis (PGD). Great controversy has taken place, and will go on, regarding the disclosure of unexpected but significant "incidental findings" from WES or WGS carried out for specific clinical purposes [4]. Furthermore today there is the technical feasibility of all newborns having their genome sequenced and screened for either childhood disorders or adult-onset conditions [5].

Advances in genetics attract great media attention, and this has brought the ethical debate to a wide public scenario. Issues about insurance, forensic science and DNA databases, patenting, gene therapy, population screening, cloning, stem cell research, and hybrids are considered to have major societal, commercial, and political importance and therefore impact clinical and laboratory practice in medical genetics. On a global scale, it is essential to safeguard fundamental principles such as privacy, confidentiality, and respect for human life at all stages and ages.

Many of the questions raised do not have easy or definitive answers, which means that there will be a great need for both public awareness and properly trained clinicians and counselors to balance the needs of their patients and families with these ethical challenges for the foreseeable future.

5. Personalized and precision medicine

The aim of personalized or individualized medicine is to use knowledge of an individual's genetic (or genomic) background relevant to the maintenance of health, prevention, and treatment of diseases as a routine part of medical care.

During the past 10 years, many examples of stratified medicine have blossomed, where the therapy of a particular disease is dependent on the germline or somatic variants patients may carry. These examples include monogenic rare diseases where a different treatment is recommended for patients with some types of germline mutations in a specific gene, such as *CFTR* gene mutations that cause cystic fibrosis, and the molecular therapy targeted on an actionable somatic mutation of a specific tumor, such as the *BRAF* V600E mutation in malignant melanoma. The genetic (or genomic) diagnosis is therefore a crucial step toward the most appropriate treatment and/or prevention, what is so-called clinical utility or actionability [6, 7]. Recent initiatives are focusing on improving health outcomes through precision medicine especially in Oncology [8] (**Table 1**). This is a multidisciplinary integrated approach that analyzes human samples and personal data to improve health care through increased precision in the knowledge of mechanisms of both disease and drug response.

Personalized genomic medicine (PGM) is only one component of precision medicine, which means, in a broadest sense, it requires clinical care providers to combine genomic information with other types of information, such as biochemical or physiological testing results, neurodevelopmental history, environmental exposures, and psychosocial experiences. The most important goal is provide more

Tumor(s)	Genes	Clinical application(s)
Melanoma	*BRAF, CDKN2A, KIT*	Therapy; risk assessment; inherited susceptibility
Breast and ovarian cancer	*BRCA1, BRCA2*	Therapy; inherited susceptibility
Colorectal cancer	*MLH1, MSH2, MSH6, PMS2, EPCAM, BRAF, KRAS, NRAS*	Therapy; inherited susceptibility
Non-small-cell lung cancer	*EGFR, BRAF, KRAS, ERBB2, RET, MET, ALK, ROS1*	Therapy
Myelodisplastic syndrome	*TP53, GATA2, JAK2, ASXL1, ETV6, RUNX1, SF3B1, EZH2*	Diagnosis; risk stratification
Acute myeloid leukemia	*KIT, CEBPA, FLT3, NPM1*	Risk stratification

Table 1.
Genetic/genomic profiling for selected malignancies (adapted from [8]).

precise diagnosis, genetic counseling, management, prevention, and therapy. This effort has already got started, but plenty of work still needs to be done before PGM becomes integrated into medicine itself.

Conflict of interest

The author declares no conflict of interest.

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Reproductive and Prenatal Genetics

The Genetic and Biochemical Blueprint of Endometrial Receptivity: Past, Present, and Future Factors Involved in Embryo Implantation Success

Almena López-Luna, Dinorah Hernández-Melchor,
Leticia Ramírez-Martínez and Esther López-Bayghen

Abstract

In the field of assisted reproductive technology, endometrial receptivity is a crucial aspect that affects implantation rates in *in-vitro* fertilization procedures; in fact, impaired endometrial receptivity has been identified as the rate-limiting step for favorable pregnancy outcomes once factors regarding embryo quality have been optimized. The endometrium is a dynamic tissue that undergoes proliferative and secretory changes in each menstrual cycle, acquiring a short and transient period of embryo receptivity known as the Window of Implantation. Precise embryo-endometrial synchrony is necessary to achieve a successful pregnancy, and it involves complex and multifactorial processes related to morphological, biochemical, and genetic changes. On that behalf, defining the receptive window of each patient for personalized embryo transfer is a current goal. Here, we review different indicators of endometrial receptivity throughout the menstrual cycle, spotlighting the opening of the window of implantation: classical histological and biochemical markers, genetic factors, leading-edge transcriptomic signatures and miRNA profiles, and novel features such as the microbiome and secretome. Understanding the molecular mechanisms behind endometrial receptivity will facilitate the optimization and improvement of infertility treatments.

Keywords: endometrial receptivity, embryo implantation, menstrual cycle, window of implantation, decidualization

1. Introduction

The field of assisted reproductive technology (ART) has grown significantly in use and understanding over the past few decades, nevertheless, the rates of successful pregnancies in *in-vitro* fertilization (IVF) procedures are still relatively low. Impaired endometrial receptivity (ER) has been identified as the rate-limiting step for favorable pregnancy outcomes once all other factors, including the acquisition and selection of the best quality embryo(s), have been optimized. Correct and synchronized maturation of the endometrial tissue is essential for embryo
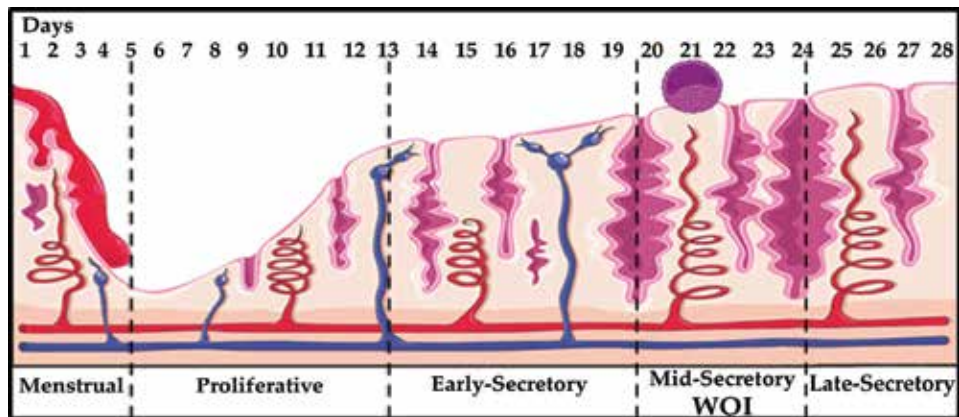
Figure 1.
Endometrial dynamics throughout the menstrual cycle. This picture was modified from Servier Medical Art under the Creative Commons License 2018.

implantation [1, 2]. The endometrium is a dynamic tissue that undergoes proliferative and secretory changes in each menstrual cycle. Throughout most of this cycle, the endometrium remains "non-adhesive" to embryos and it only acquires a short and transient period of embryo receptivity known as the "window of implantation" (WOI) [3, 4]. In humans, during a natural cycle, the endometrium becomes receptive 6 to 8 days after ovulation and it remains receptive for approximately 24–48 h, this time is assumed to occur between days 20 and 24 of a regular menstrual cycle [2, 5] (**Figure 1**). The cyclic fluctuations of the endometrium are coordinated by the ovarian hormones estrogen (E2) and progesterone (P4); a finely balanced signaling process mediated by these hormones defines the WOI. The menstrual cycle is divided in two phases: proliferative or follicular, and secretory or luteal [6]. During the proliferative phase (PP), E2 from the maturing follicle allows the elongation of the spiral arteries and the proliferation of endometrial stromal cells (EnSCs) and glands [7]. Afterwards, during the secretory phase (SP), P4 from the corpus luteum induces secretory changes; the endometrium thickens and it acquires a receptive phenotype that will support blastocyst attachment [8–10]. During the Mid-Secretory Phase (MSP) circulating P4 induces EnSCs to undergo decidualization [11]. Decidualization is the transformation of the endometrial stroma into a dense cellular matrix known as the decidua, this process initiates during the SP in the stroma and, if pregnancy occurs, it progresses into the development of the decidua which will in turn form the maternal placenta [12]. The optimization of the endometrium to support embryo implantation is a complex and multifactorial process that involves morphological, biochemical, and genetic changes [13]. ER is a key aspect that affects implantation rates in IVF procedures considering that a precise embryo-endometrial synchrony is completely necessary to achieve a successful pregnancy [4, 14]. Thus, understanding the molecular mechanisms behind ER will facilitate the optimization and improvement of infertility treatments.

2. Factors involved in endometrial receptivity

2.1 Evaluation of endometrial morphology for receptivity assessment

Morphological changes during the endometrial cycle generate markers that have been used over decades to assess receptivity, such as histological evaluation of a biopsy and ultrasound examination of the endometrium. Endometrial biopsies are now

considered to provide little clinically relevant information [15, 16]. Additionally, the formation of pinopodes was thought to show potential as a clinical marker to assess ER [17]. However, the presence of pinopodes was demonstrated not only during the WOI but also in the post-receptive endometrium, precluding in this way its use as a marker of ER [17, 18]. On the other hand, ultrasound examination is a routinely used technique in IVF procedures [9, 19]. This non-invasive technique is based on the interpretation of a medical ultrasound of the endometrium. Various ultrasonographic parameters have been proposed as pregnancy predictors, such as endometrial thickness, volume, and blood flow patterns. The most commonly used is endometrial thickness [20, 21]. Due to differences in stimulation protocols, sonographic approaches, and difficulties in obtaining a standard sagittal view of the uterus, discrepancies in the cut-off value of endometrial thickness to achieve pregnancy arise [22]. Generally, it is considered that a minimum of 6–8 mm in endometrial thickness is necessary for a successful pregnancy [23–25]. Nevertheless, case reports have described pregnancy establishment despite an endometrial thickness of no more than 4 mm [26, 27]. Three-dimensional (3D) sonography assesses ER by considering endometrial thickness, volume, and angiogenic dynamics. The endometrial volume of fewer than 2 ml has been shown to decrease pregnancy rates significantly [19, 28, 29]. Another evaluated criterion is an endometrial pattern, which can be classified as triple-line, intermediate, or homogenous [30]. Among these, the triple-line pattern has been suggested to reflect ER [24, 31] broadly. Finally, the impact of ovarian stimulation on ER has yet to be determined. Abnormal hormone concentrations the due to stimulation protocols during IVF might affect endometrial morphology and thereby ER [32]. Comprehensively, although morphological elements are important components of receptivity, there is still no consensus on the extent in which they can be used as WOI predictors.

2.2 Genetic factors involved in ER

Endometrial genetic abnormalities can lead to implantation failure due to dysregulation of critical processes such as trophoblast invasion and angiogenesis. Here, we discuss common genetic abnormalities that have been analyzed to determine their role in implantation failure (**Table 1**). Parental chromosomal abnormalities such as mutations and translocations should be considered relevant in the efficacy of improving reproductive outcome.

2.2.1 Angiogenetic factors: vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS), TP53 tumor suppressor (TP53), murine double minute 2 (MDM2), herpes virus-associated ubiquitin-specific protease (HAUSP)

Successful pregnancy is dependent on adequate placental circulation and fetal vasculature. The development of a normal vascular network during implantation, embryo development, and placentation requires cooperation between different cell types and various growth factors. *VEGF* is a potent angiogenic factor that plays an essential role in embryo implantation/development. Four *VEGF* polymorphisms have been reported to affect VEGF activity and expression increasing aberrations in vascular formation and/or function. The polymorphism −1154G/A located in the promoter region has been associated with RPL [33], RSA [34, 35], and RIF [36, 37]. Moreover, a meta-analysis showed that genotypes −2578C/A, −634G/C, and 936C/T increase the risk of RSA as well [38]. Furthermore, *eNOS,* which is expressed in the terminal chorionic villous vessels, is important for vascular nitric oxide (NO) production to supply nutrients to the fetus. Only the eNOS Glu298Asp polymorphism has been shown to be significantly associated with RPL [39]. Additionally, successful trophoblast invasion requires the induction of paracrine apoptotic reactions to

Gene	Polymorphism	rs code	Relevance	References
APOE			Heterozygous genotype is more frequent in women with RPL	[33]
eNOS	VNTR (B/A)		Association to the risk of RPL	[77]
			No associated	[78]
	Glu298Asp		Homozygote genotype T/T is associated with risk of IRM	[39]
ESR1	IVS1-397T>C	rs2234693	Related to unknown thin endometrium in which P allele may be the risk and X allele its guard factor	[44]
	IVS1-351A>G	rs9340799		
F2	G20210A	rs1799963	No association	[58, 60, 62, 63]
			Heterozygous genotype is more frequent in women with RSA in the first trimester [57] and women with RPL [42]	[42, 57]
F5	G1691A, Leiden	rs6025	No association	[57, 58, 62, 63]
			More frequent in women with RIF	[60]
	H1299R (R2)		No association	[58, 62, 63]
	Y1702C		No association	[58, 62, 63]
F8	V34L		More prevalent in women with RPL	[42, 58]
			No association	[62, 63]
FGB	G-455A	rs1800790	No association	[57, 58, 62, 63]
GPIIIa	C1565T		No association	[57]
HAUSP	rs1529916 G/A	rs1529916	Allele A is associated with RIF	[40]
HPA1	HPA1 a/b (L33P)		No association	[58, 62, 63]
LIF	C715A		No associated	[72]
	G3400A		More frequent in nulligravid women	[72, 73]
	G3424A		No associated	[72]
	T1414G	rs929271	G/G genotype is associated with RIF	[40, 74]
MDM2	T309G	rs2279744	Allele G is associated with RIF	[40]
MTHFR	A1298C	rs1801131	No association	[58, 62–64]
	C677T	rs1801133	More frequent in women with unexplained infertility [64], RPL [42, 58], and RSA [60]	[42, 58–60]
			No association	[57, 62, 63]
MUC1	VNTR		Women with unexplained infertility might have susceptibility to implantation failure due to small *MUC1* allele size	[67]
			No association	[68, 69]
MUC4	VNTR		No association	[70]
PAI-1	4G/5G	rs1799889	More prevalent in women with RIF [63] and RPL [42, 58]	[42, 58, 63]
			No association	[62]

Gene	Polymorphism	rs code	Relevance	References
PR	H770H-C/T		No association	[54]
	G/T—Val660Leu	rs1042838	More prevalent in women with unexplained infertility	[65]
	V660L		No association	[54]
PT53	Codon 72 Pro	rs1042522	Homozygote genotype is associated with RPL [41, 75], IRM [76] and RIF [37, 40]	[37, 40, 41, 75, 76]
	Codon 72 Arg	rs1042522	Homozygote genotype is associated with RIF	[75]
PTGS2	G-765C	rs20417	Association with implantation failure susceptibility	[55]
TFF3	rs225361 A/G	rs11701143	Homozygous genotype is associated with less live births before their first spontaneous abortion	[61]
	rs225361 A/G	rs11701143	No association	[66]
	rs11701143 T/C	rs225361	Associated with idiopathic RSA	[61]
	rs11701143 T/C	rs225361	No association	[66]
	rs225439 G/A	rs225439	No association	[61, 66]
	ros533093 C/T	rs533093	No association	[61, 66]
	rs77436142 G/C	rs7743614	No association	[61, 66]
VEGF	G-1154A	rs1570360	Homozygote A/A genotype associated with RSA [34, 35], RPL [33], and RIF [36, 37]	[33–37]
			No associated	[71]
	C-2578A	rs699947	No associated	[34]
	G-634C	rs2010963	No associated	[34]
	C936T	rs3025039	No associated	[34]

Probably implicated mutations in implantation failure, studies are listed even when no association was found, rs code is mentioned whenever it is reported.

Table 1.
Genetic abnormalities involved in implantation failure.

secrete proteases capable of digesting the endometrial extracellular matrix (ECM) [36]. TP53 is a potent regulator of apoptosis, cell cycle, angiogenesis, and embryonic development. A TP53 polymorphism at codon 72, encoding either proline or arginine, was reported to alter the TP53 activity and affect human fertility [40]. The Arg72 variant has been shown to induce higher apoptotic activity than Pro72. Therefore, Pro72 variant might cause inadequate trophoblast invasion, increasing the risk of RPL [41] and RIF [37, 40]. In this manner, women with Pro/Pro genotype have a higher risk of RPL than women with the Arg/Arg or Arg/Pro genotypes. Following, MDM2 and HAUSP regulate TP53. MDM2 binds to TP53 to degrade it through poly-ubiquitination, blocking its ability to function as a transcription factor. The *MDM2* SNP309 is a functional SNP that increases MDM2 expression levels and attenuates TP53 pathway. HAUSP, on the other hand, acts as a specific deubiquitinase for TP53, the A allele has a significant association with infertility in young patients (<35 years) but not in the older patients, similarly to the *MDM2* SNP309 G allele. Those observations suggest that *MDM2* and *HAUSP* may be involved in the regulation of human fertility through the regulation of TP53 [40].

2.2.2 Apolipoprotein E (APOE)

Due to the increase in total cholesterol levels during pregnancy, *APOE* plays a crucial role in lipid metabolism. *APOE* has three alleles in the long arm of chromosome 19 at position 13.2: ε2, ε3, and ε4. Individuals harboring the allele ε4 have higher cholesterol levels than the ones carrying the ε3/ε3 allele, whereas levels in those with the ε2 allele are lower [42].

2.2.3 Estrogen receptor α (ESR1)

ESR1 is a ligand-activated transcription factor essential for sexual development and reproductive function; its dysregulation leads to the development of various diseases such as cancer, cardiovascular disease, and inflammation, among others [43]. Due to alternative splicing of mRNA, it possesses three isoforms: ERαΔ3, ERα36, and ERα46 [43]; in a study performed by Yuan and Le [44], the polymorphisms rs2234693 and rs9340799 were related to the uncommonly thin endometrium.

2.2.4 Leukemia inhibitor factor (LIF)

LIF is an important implantation factor that promotes proliferation, invasion, and differentiation; its expression is regulated by the transcription factor tumor protein TP53 (TP53). Few studies have found a correlation between LIF gene polymorphism and reproductive capacity, Kang et al. demonstrated that SNP in the 3'UTR of the LIF (rs929271) gene is associated with infertility [40].

2.2.5 Mucin 1 (MUC1), Mucin 4 (MUC4)

MUC1 is an anti-adhesion molecule secreted by human endometrial epithelium, it has been suggested that its expression prevents the adherence of blastocyst to the endometrium. Interestingly, MUC1 must be locally removed in a paracrine fashion at the implantation site during the WOI to allow contact between the embryo and the endometrium, making it an important factor in determining ER [45–48]. *MUC1* is a highly polymorphic gene that differs in the size of the region carrying the O-glycosylation sites: the variable number tandem repeat region (VNTR), which can go from 20 to 125 repeats [49]. Similarly, MUC4 is a greatly expressed mucin in endometrial epithelium [50], its gene is highly polymorphic and it contains a VNTR region that can go from 145 to 395 repeats [51]. Although its role in human infertility has not been fully explored, studies in other species have suggested that it plays a role in embryo implantation [52, 53].

2.2.6 Progesterone receptor (PR)

The PROGINS complex are three mutations in the *PR* gene that may be associated with unexplained infertility and implantation failure: a 306 bp insert in intron G of the dT2 allele in *PR*, the mutated alleleV660L, and guanine to thymine substitution in exon 4, resulting in a valine to leucine change in the hinge region of PR, and a cytosine to thymine substitution at exon 5 [54].

2.2.7 Prostaglandin-endoperoxide synthase 2 (PTGS2)

PTGS2 is a key enzyme involved in the conversion of arachidonic acid to prostaglandins (PGs). The −765G>C SNP mutation in the promoter region of *PTGS2* upstream the transcriptional start site in the putative Sp1 site can cause alterations in Sp1 binding

[55]. Accordingly, the hypermethylation of the NF-IL6 site within the *PTGS2* promoter results in elevated gene expression in eutopic endometrium in endometriosis [56].

2.2.8 Thrombolytic factors: coagulation factor II (F2), coagulation factor V (F5), coagulation factor XIII a chain (F13A1), methylenetetrahydrofolate reductase (MTHFR), plasminogen activator inhibitor-1 (PAI-1)

Thrombophilia, the predisposition for thrombosis, has been shown to be a risk factor for successful pregnancy due to impaired vascularization at the time of implantation. Therefore, the possible association between early pregnancy loss and polymorphisms at coagulation factors and thrombolytic genes responsible for inherited or acquired thrombophilia has been investigated. The coagulation factor II SNP G20210A in the 3′-untranslated region of *F2* causes elevated prothrombin in plasma, leading to enhanced blood coagulation [57]. Furthermore, factor V Leiden mutation, G1691A, is a single nucleotide substitution in the *F5* gene that results in reduced clearance of factor Va due to its blocked inactivation by activated protein C, increasing the risk of thrombosis [42, 57]. Also, factor XIII V34L polymorphism is a guanine to thymine substitution in exon 2 of *F13A1* that leads to a valine for leucine change in residue 34; this SNP leads to reduced susceptibility to fibrinolysis and influences fibrin degradation [57]. Moreover, MTHFR is the rate-limiting enzyme in the methyl cycle. C677T polymorphism causes a substitution of valine for alanine, resulting in a thermolabile variant of the enzyme with reduced catalytic activity; combined with the SNP AC1298C, it is associated with hyper-homocysteinemia, a risk factor for venous and arterial thrombosis [42, 58–60]. Additionally, PAI-1 is a key regulatory element in the fibrinolysis cascade, it is believed to control proteolysis and remodeling of maternal tissue during trophoblast invasion. The 4G/5G polymorphism is located 657 bp upstream from the start site of transcription within the *PAI-1* promoter and results in an allele with decreased transcriptional activity [42].

2.2.9 Trefoil factor 3 (TFF3)

TFF3 is a mucin-associated peptide co-expressed in mucus cells that acts as a mitogen to promote epithelial cell migrations and mediates epithelial repair after damage. The SNP rs11701143 is located in the promoter region of *TFF3* within the regulatory region of the transcription binding site, whereas rs225361 is an intron variant located within a regulatory region. The exact function of both SNPs remains to be elucidated [61].

2.3 Immunological factors contributing to ER

The immune system plays a major role in the process of implantation and pregnancy maintenance [62]. During decidualization, endocrine processes transform uterine fibroblasts into cells that can produce hormones, growth factors, and matrix components to support embryo implantation [63, 64]. Furthermore, tolerance of the immune maternal system is required in pregnancy to avoid rejection of the semi-allograft or allograft embryo and for its successful implantation [65]. The decidua is a privileged site for immune tolerance; a large number of molecules and immune cell types participate in this process, leukocytes, macrophages, T lymphocytes, and dendritic cells comprise around 30 to 40% of the cells within the decidual stroma in early pregnancy. Among leukocytes, uterine natural killer (uNK) cells are activated and they significantly increase during decidualization (65–70%) [66, 67]. Increases in uNK cells denote three main functions in the endometrium: regulation of placental and trophoblast growth by cytokines [68, 69], local immunomodulation [70, 71], and

control of trophoblast invasion [69]. Furthermore, trophoblast cells play a major role in immune tolerance since these cells do not express major histocompatibility complex (MHC) class I (HLA-A and HLA-B) or class II molecules; ensuring that maternal T cells with αβ receptors cannot mount a classic cytotoxic attack against fetal paternal alloantigens. The trophoblast also protects itself by expressing Fas ligand (Fas L), which is important in the elimination of maternal reactive T cells by apoptosis induction [72–74]. Other important component of this process is T-regulatory (Treg) cells; these cells are essential for immunosuppression, prevention of autoimmunity, and maternal tolerance to the fetus [75–78]. Treg cells have been shown to be locally enriched in decidua during early normal pregnancy [79]. Furthermore, Forkhead box P3 (Foxp3) is a master regulator of Treg cell development, function, and differentiation [80]. Expression of *FOXP3* was reduced approximately two-fold in endometrial biopsies of infertile women, implicating that the impaired differentiation of uterine T-cells into the Treg phenotype is a key determinant of fertility [81, 82]. On the other hand, helper T cells (CD4+) facilitate embryo implantation by regulating endometrial differentiation; they secrete interleukins and interferons that establish the implantation microenvironment. Successful pregnancy is dependent upon Th1/Th2 balance [83]. While Th1 cytokines are harmful for pregnancy, Th2 cytokines favor fetal growth and regulate uterine expression of fatty acid amide hydrolase (FAAH), LIF, and trophoblast release of human chorionic gonadotropin (hCG), which are known to play important roles during implantation [84–86]. Piccinni et al. demonstrated that T cells from decidua of women with a miscarriage show predominantly Th1-type cytokines with decreased Th2-type [84, 87, 88]. Finally, P4 and E2 mediate the downregulation of the maternal immune system [89]. P4 stimulates decidual proliferation; therefore, pregnancy results in an upregulation of P4 receptors on activated lymphocytes among placental cells and decidual CD56+ cells. In the presence of sufficient P4, these cells express progesterone-induced blocking factor (PIBF), a mediator that exerts substantial anti-abortive activities.

2.4 Biochemical markers involved in ER

We review molecular markers involved in the decidualization process that could be suitable as makers to assess ER.

2.4.1 Homeobox A10 (HOXA10)

HOXA10 is a transcription factor member of the homeobox family, known to be involved in the genetic control of embryonic development and in the regulation of the adult female reproductive tract [12, 90]. HOXA10 regulates downstream target genes that lead to endometrium development and receptivity acquisition [91], such as insulin-like growth factor binding protein 1 (*IGFBP1*) [12, 92, 93], genes of the Wnt pathway (reviewed by Sonderegger et al. [94], integrin β3 (*ITGB3*) [12, 90, 92], and empty spiracles homolog 2 (*EMX2*) [12, 95]. HOXA10 is regulated by P4 in a dose-dependent manner [12, 90, 91]. The expression of HOXA10 is low during the PP and it rapidly increases in the MSP [12, 90]. The diminished expression of HOXA10 in endometria of women with recurrent pregnancy loss (RPL) [96], adenomyosis [97], endometriosis [92], polycystic ovary syndrome (PCOS) [98], and idiopathic infertility [99] indicates that this gene could be essential for fertility [12].

2.4.2 Heparin-binding epidermal growth factor-like growth factor (HB-EGF)

HB-EGF is a member of the epidermal growth factor (EGF) family, it is expressed in the human uterus at the time of implantation and its expression is under steroidal hormone control [100–103]. The transmembrane form is associated

with cell adhesion and migration, it allows communication with the blastocyst by acting as a chemoattractant [101, 104]. HB-EGF expression is low in the PP and increases in the ESP immediately prior to the WOI, after which its levels decrease [101–103, 105]. Also, mRNA levels are low in pregnant endometrium and high in placental tissues at an early stage of development, suggesting that the HB-EGF ligand not only potentiates the health and survival of the peri-implantation embryo, but also induces the progression of its development [104]. HB-EGF stimulates epithelial expression of key endometrial proteins that are important biomarkers of the WOI, including LIF, HOXA10, and ITGB3 [100].

2.4.3 Leukemia inhibitory factor (LIF)

LIF is a member of a cytokine family with functional redundancy that includes interleukin 6 (IL6), oncostatin (OSM), ciliary neurotrophic factor (CNTF), and cardiotrophin 1 (CT1). They regulate proliferation, differentiation, and cell survival in different cellular systems [106]. LIF acts on cells by binding to the heterodimeric LIF receptor (LIFR), which consists of two transmembrane proteins, LIFR and glycoprotein 130 (gp130). LIFR activates several signaling pathways including the JAK/STAT, MAPK, and P13-kinase pathways, whereas gp130 participates in the activation of STAT1, STAT3, and STAT5B [107]. LIF induces the expression of cytokines and other regulatory molecules that could serve to regulate preimplantation development and embryo implantation [106–108]. LIF is one of the most important cytokines for receptivity during the WOI, the expression of LIF and LIFR reaches its highest level during the WOI in the MSP, LSP, and in early pregnancy in both surface and glandular epithelial cells under the influence of P4 [106, 107, 109, 110]. LIF can also be detected in decidual leukocytes, which are abundant at the implantation site; interestingly, LIF expression is low in women with unexplained infertility [106, 107, 111]. LIF also plays a crucial role in the regulation of fetal-maternal interactions during pregnancy, this cytokine mediates uterine receptivity through autocrine/paracrine interactions by binding to LIFR on the luminal epithelium to permit blastocyst attachment [106], but also regulates trophoblast function and vascular formation in the placenta [109].

2.4.4 Integrin β3 (ITGB3)

Integrins are ubiquitous cell adhesion molecules involved in maintaining normal tissue morphology and participate in cell–cell and cell-substrate interactions [100, 110, 112, 113]. In the human endometrium, integrins are involved in early embryo-endometrial interactions [90]. ITGB3 subunit is present after the ESP and its expression extend into the pregnancy [112, 114]. It has been reported that healthy fertile women show higher ITGB3 expression than patients with unexplained infertility [46, 96, 113–115]. Moreover, its dysregulation appears to characterize two distinct pathophysiological conditions that involve distinct mechanisms of defective ER: Type I and Type II. Type I defect is an out-of-phase endometrium with negative ITGB3 subunit expression, portrays a shifted WOI, and hormonal inadequacy or responsiveness is implicated, on the other hand, Type II defect is an "in-phase" endometrium with negative ITGB3 subunit expression and connotes the complete loss of the WOI. Furthermore, ITGB3 is expressed in EnSCs and endometrial glands with the highest levels in the MSP to LSP, suggesting a role in the regulation of endometrial function and implantation [115, 116]. Due to its temporal distribution and the effects of implantation when it is not present, ITGB3 is a useful molecular marker to assess ER. ITGB3 is regulated in the endometrium through a molecular mechanism via sex steroid signaling where HOXA10 acts as an intermediary [90, 96].

Other identified markers that are important for decidualization in the human endometrium include *PR*, particularly its encoded isoform progesterone receptor A (PR-A), homeobox A11 (*HOXA11*), *PTGS2*, *MUC1,* and interleukin 11 receptor (*IL11R*) [12].

2.5 Transcriptomic signature to determine the WOI

Microarray technology has been widely used to determine the transcriptomic profile of the endometrium by analyzing the expression of large batches of genes at different stages of the menstrual cycle. The most representative and commercially available test in this regard is perhaps the Endometrial Receptivity Array (ERA), developed in 2009 by Diaz-Gimeno et al., this test identifies the unique transcriptomic signature of the receptive endometrium by analyzing 238 differentially expressed genes, predicting the WOI for personalized embryo transfer (pET) [108]. Various research groups have analyzed changes in gene expression during the different phases of the endometrial cycle using microarray-based technologies [117–120], however, due to differences on results, unanimity about the main genes to be analyzed to determine the WOI has not been reached. Factors that contribute to the disagreement among studies results include differences on experimental design, utilized probes, sample acquisition day, sample size, collection method, and the application of distinct statistical analyses. Nevertheless, some genes have been reported to be expressed similarly in more than one work, here, we present a compilation of the expression profiles of those candidate genes in the human endometrium (**Table 2**).

2.6 miRNAs involved in ER

Micro-RNAs (miRNAs) are small, single-stranded, non-protein-coding RNA sequences of ~18–25 nucleotides in length that play an important post-transcriptional regulatory role in gene expression [121, 122] by targeting mRNAs for cleavage or transcriptional repression [123]. More than two decades have passed since the initial discovery of miRNAs in *Caenorhabditis elegans* by Lee et al. [124]; since then, great progress has been made in the understanding of miRNAs: what they are, how are synthesized, how regulate gene expression, and how they are involved in the formation and progression of pathological disorders. Extracellular miRNAs have been ubiquitously detected in body fluids [125]. Therefore, the presence and stability of miRNAs in biological fluids have advocated their potential as non-invasive biomarkers. Nevertheless, the identification of reliable miRNA biomarkers with reproducible profiles has been a challenge, and their diagnostic promise has remained a work in progress since they have still not entered the clinical field [126]. Nonetheless, given that miRNAs are differentially expressed in the endometrium across the menstrual cycle [127–131], several studies have been conducted to explore their role in ER [131–136]. **Table 3** presents a summary of these studies.

2.7 The endometrial secretome as a potential tool to ascertain ER

The aim to develop alternative non-invasive strategies to provide accurate receptivity assessment has drawn assiduity to the endometrial secretome, which is based in the identification of factors secreted by cells or tissues at a particular time in either physiological states or pathological conditions [137], including proteins, lipids, and metabolites. Therefore, the analysis of differentially present molecules in the uterine cavity at different time points of the menstrual cycle could potentially help to identify the WOI and to diagnose uterine pathologies. Sample collection of endometrial fluid (EF) collection in the peri-implantation period is an easy procedure performed with minimally invasive tools that could easily be implemented

Gene	Functional category	Expression profile		References
Annexin 4 [*ANX4*]	Apoptosis	PP to MSP	↑	[73, 86, 91–95]
		ESP to MSP	↑	
		MSP to LSP	↓	
Apolipoprotein D [*APOD*]	Cholesterol transport and trafficking	PP to ESP	↑	[85, 92, 96]
		ESP to MSP	↑	
Claudin 4 [*CLDN4*]	Cell adhesion	ESP to MSP	↑	[73, 85, 93, 94, 96, 97]
Decay-accelerated factor [*DAF*]	Immune modulators/ cytokines	ESP to MSP	↑	[85, 93, 94, 98]
Dickkopf-1 [*DKK1*]	Regulation of WNT signaling pathway	PP to ESP	↑	[73, 85, 92, 94, 96, 97, 99]
		ESP to MSP	↑	
		ESP to LSP	↓	
Endothelin 3 [*EDN3*]	Vasoactive substances	ESP to MSP	↓	[73, 92, 93, 100]
Growth arrest and DNA-damage-inducible protein [*GADD45*]	Cell cycle	PP to MSP	↑	[73, 92–94, 97, 101]
		ESP to MSP	↑	
		MSP to LSP	↑	
Glutathione peroxidase 3 [*GPX3*]	Response to stress and oxidoreductase activity	ESP to MSP	↑	[73, 86, 96]
Homeobox A10 [*HOXA10*]	Transcription factor	ESP to MSP	↑	[55, 64, 94, 102, 103]
		MSP to LSP	↑	
Inhibitor of DNA binding 4, dominant negative helix–loop helix protein [*ID4*]	Anatomical structure development	ESP to MSP	↑	[73, 92–94, 104]
Insulin-like growth factor binding protein 1 [IGFBP-1]	Anatomical structure development	ESP to MSP	↑	[73, 94]
		MSP to LSP	↑	
IL15 precursor [*IL15*]	Immune response	ESP to MSP	↑	[73, 85, 92–94, 96]
Mitogen-activated protein kinase kinase kinase 5 [*MAPKKK5*]	Signal transduction	PP to ESP	↑	[92, 101]
Matrix metalloproteinase 26 [*DAMMP26*]	Tissue remodeling and blastocyst invasion	MP to LPP	↑	[87, 94]
		PP to ESP	↑	[73]
		MSP to LSP	↓	
Msh homeobox homologs 1,2 [*MSX1*]	Anatomical structure development	ESP to MSP	↓	[73, 88, 91, 93, 96]
Natural cytotoxicity-triggering receptor 3 [*NCR3*]	Immune response	ESP to MSP	↑	[86, 87]
		MSP to LSP	↑	
		LSP to MP	↑	
		MP to PP	↓	
Olfactomedin 1 [*OLFM1*]	Anatomical structure development	PP to ESP	↓	[73, 88, 92, 93]
		ESP to MSP	↓	
Sex-determining region Y-box 4 [*SOX4*]	Apoptotic pathways	PP to ESP	↓	[87, 88]
		MSP to LSP	↑	
		MP to LPP	↑	
Osteopontin [*SPP1*]	Cell adhesion	PP to SP	↑	[73, 85, 91–93, 96, 97, 101]
		ESP to MSP	↑	
Tissue inhibitor metalloproteinase 3 [*TIMP-3*]	Degradation of the extracellular matrix	PP to ESP	↑	[73, 94, 95]
		ESP to MSP	↑	
		MSP to LSP	↑	

Genes expressed in human endometrium and its expression profiles at the different phases of the endometrial cycle. A compilation of the genes exhibiting the same expression profile in more than one work is presented, regardless of the differences among studies.

Table 2.
Expression profiles of genes involved in ER.

Reference and relevance	Methodology	Results	Targets
[132] Shows distinct miRNA profiles in LPP and MSP	**Samples:** Endometrial biopsies LPP [n = 4] and MSP [n = 4] **Technique:** Microarray Technology	49 differentially expressed miRNAs: 12 ↑ in the MSP [miR-214, 503, 134, 450, 382, 376A, 369-5p, 222, 370, 542-3p, 105, and 127] and 12 ↓ in LPP [miR-210, 193a-3p, 345, 29b, 29c, 30b, 204, 203, 582-5p, 30d, 200c, and 31]	**Predicted:** Cell cycle pathways in the LPP. Wnt signaling pathway in the PP. **Validated:** Decreased transcripts of predicted targets [cyclins, CDKs, and E2F3]
[134] Evaluates differentially expressed miRNAs in RIF-IVF patients	**Samples:** Secretory endometrium; 12 fertile women versus 11 women with RIF **Technique:** TaqMan miRNA array cards	13 differentially expressed miRNAs: 10 ↑ [miR-23b, I45, 99a, 27b, 652, 139-5p, 195, 342-3p, 150, and 374b] and 3 ↓ [miR-32, 628-5p, and 874]	**Predicted:** Wnt signaling, adherents junctions, p53 signaling, cell adhesion, and cell cycle pathways. **Validated:** Decreased transcripts of N-cadherin, H2AFX, NTN4, and SFRP4. Wnt and cell cycle pathways ↓ in RIF-IVF
[154] Makes comparison between natural vs. stimulated cycles. Suggests that ovarian stimulation may shift the WOI	**Samples:** Infertile women. Receptive [LH + 7, n = 5] vs. prereceptive [LH + 2, n = 5] in natural cycles. Receptive [hCG + 7, n = 5] vs. prereceptive [hCG + 4, n = 5] in stimulated IVF cycles **Technique:** Next-gen seq., 626 miRNAs evaluated	20 differentially expressed miRNAs in natural cycles: 8 ↑ [miR-30d, 30b, 30b, 31, 21, 193a-5p, 193a3p, 203] and 12 ↓ [miR-33a, 452, 125b, 455-3p, 455-5p, 483-5p, 143, 100, 504, 424, 424, 503]. 22 differentially expressed miRNAs in stimulated cycles: 19 ↑ [miR-187, 708, 433, 320a, 320b, 34c-5p, 320c, 320d, 485-5p, 574-5p, 375, 23b, 423-5p, 193b, 34b, 503, 424, 455-5p, 483-5p] and 3 ↓ [miR-886-5p, let-7f, let-7a]	**Predicted:** Cell cycle, transport, cell adhesion, cell death, and metabolism
[136] Provides miRNA signature of EnSCs during decidualization *in vitro*	**Samples:** Endometrial samples on oocyte retrieval day from healthy ovum donors [n = 50]. EnSCs [n = 20] isolated and cultured **Technique:** miRNA PCR array, 704 miRNAs + specific miRNA-200b primers evaluated	43 differentially expressed miRNAs: 26 ↑ [miR-95, 888, 936, 1185, 518f, 548 k, 593, 486-5p, 29c, 449b, 300, 371-5p, 1224-3p, 891a, 365, 541, 409-5p, 33b, 154, 376a, 133a, 218–2, 22, 614, 369-3p, 185] and 17 ↓ [miR-146a, 155, 181b, 181a, 135b, 181d, 200c, 141, 182, 429, 483-3p, 200a, 96, 183, 9, 30a, 126] miR-95 ↑ by P4 and E2 + P4; miR-96 and miR-135b both ↓ by E2 or P4	**Predicted:** Growth factors, interleukins, ECM remodeling enzymes. • Top pathways by ↓ miRNAs: axon guidance, adherents junction, actin cytoskeleton regulation, ErbB [EGFR] signaling, and renal cell carcinoma. • Top pathways by ↑ miRNAs: actin cytoskel-eton regulation, adherent junction, axon guidance, Wnt signaling, and MAPK
[133] Provides miRNA signature of fertile human endometria: receptive vs. prereceptive	**Samples:** Receptive MSP [LH + 7, n = 4] vs. prereceptive ESP [LH + 2, n = 5] endometrial biopsies from 9 healthy fertile women **Technique:** Microarray, 723 human and 76 human viral miRNAs evaluated	4 significantly expressed different miRNAs in receptive samples: 2 ↑ [hsa-miR-30b and 30d] and 2 ↓ [hsa-miR-494 and 923] Suggests 12 genes that could serve as a new panel for ER: CAST, CFTR, DPYSL2, FI IR, FGFR2, LIF, MTFI, NPAS2, P4HA2, PPARGCIA, TACC2, RAB40B	**Predicted:** Transcription, cell proliferation, and apoptosis. Involvement in pathways such as axon guidance, Wnt/β-catenin, ERK/MAPK, TGF-β, p53 and leukocyte extravasation. They identified *SEPT7, CRMP1, SLC44A1, HES1, FXR2,* and *TNF144B* as genes that interact with genes *MIR30B* and *MIR30D*

Reference and relevance	Methodology	Results	Targets
[130] Differential miRNAs across cycle. Release of miRNAs into the EF and its uptake by the embryo	**Samples:** EF [n = 20] at different phases of the menstrual cycle of healthy women. Timing of sample collection: EPP, LPP, ESP, WOI, and LSP **Technique:** Microarray, 866 miRNAs evaluated	Compared with the WOI, 9 differentially expressed miRNAs were identified in the EPP, 8 in the LPP, 6 in the ESP and 4 in the LSP. MiR-30d was the most differentially secreted maternal miRNA in the EF during the WOI	**Predicted:** Cell cycle and endocrine processes
[135] Shows a significant different expression of miRNAs in the WOI of RIF patients that may contribute to impaired ER	**Samples:** Endometrial biopsies from the WOI [5–7 days after ovulation]: 7 from RIF group and 5 from control group [infertile patients that delivered after one transfer attempt] **Technique**: Microarray Technology	With a 2-fold threshold: 105 miRNAs were differentially expressed: 93 ↑ and 12 ↓. After raw signal value correction, 15 were found to be significantly different. 10 ↑ [hsa-miR-374a-5p, 145-5p, 30b-5p, 196b-5p, 199a-5p, 199b-5p, 449a, 424-5p, 125b-5p, 21-5p] and 5 ↓ [hsa-miR-1207-5p, 4306, 572, 5739, 6088]	**Predicted:** TAM analysis: miR-30 family, human embryonic stem cell regulation, epithelial-mesenchymal transition, and miRNA tumor suppressors. Network regulatory analysis: 176 miRNA-mRNA interactions. The top core mRNA were ABP1, AQP3, ASS1, and TIMP3, the top core miRNAs were has-miR-4668-5p, 429, and 5088

Studies conducted to analyze miRNA expression profiles during the menstrual cycle as potential biomarkers of the WOI. A summary of the differentially expressed miRNAs and predicted targets found in recent studies is presented. Its relevance regarding the role of miRNAs is also addressed.

Table 3.
Studies of miRNA-profiling during the WOI.

in ART procedures. Proteomics of EF has already rendered valuable information regarding ER; Casado-Vela et al. identified 803 proteins in EF aspirates using three different proteomic strategies [138]. Additionally, Boomsma et al. [139] analyzed endometrial secretions prior to embryo transfer from 210 women undergoing IVF to determine differences in cytokine profiling at the time of implantation, finding a negative and a positive association of monocyte chemo-attractant protein-1 (MCP-1) and IFN-γ-inducible 10 kDa protein (IP-10) levels and implantation, respectively. Lipidomics, on the other hand, seems to have rendered slight information on receptivity [140], nevertheless, a study performed by Berlanga et al. [141] and followed by Vilella et al. [142, 143] carried out lipidomic analyses of EF from patients at different stages of their menstrual cycle, they determined a significant increase in Prostaglandin E2 (PGE2) and Prostaglandin F2α (PGF2α) between days 19 and 21, coincident with the WOI. In a recent study performed by Durairaj, Aberkane et al. [144], the contribution of EnSCs to failed implantation was examined by analyzing the secretome profile of EnSCs cultures *in-vitro*. From there, they encountered that secretome profiles of pregnant women are less divergent in implantation-positive-cultures particularly in Day 0 (undifferentiated cells), suggesting that endometrial defects linked to reproductive failure could be more prominent in the PP, a phase that is commonly thought to be not relevant for ER studies. This research group also demonstrated that the secretome of undifferentiated EnSCs compromises blastocyst development. Finally, they determined that a deficiency of endometrial mesenchymal stem-like cells (MSCs) could lead to aberrant EnSC function and implantation failure. Overall, this study remarks the importance of progenitor cell populations in

the endometrium that supports the acquisition of receptivity and raises the prospect of screening the endometrium before the initiation of an ART procedure.

2.8 The microbiome as a novel aspect of ER

Historically, the uterus was assumed to be free of bacteria as the fetal environment was considered to be physiologically sterile [145], this notion implies that the neonate's microbiome is acquired only during and after birth. Although recent research still supports this conception [146], others have characterized upper genital tract microbiota [147–150], suggesting that the endometrial and vaginal microbiota not be identical [151]. What is more, the study by Moreno et al. [147, 152], which defined the microbiota in the EF as *Lactobacillus*-dominated (LD) or non-*Lactobacillus*-dominated (NLD), suggested that the presence of an NLD microbiota in a receptive endometrium was associated with a significant decrease in implantation, pregnancy, ongoing pregnancy, and live birth rates. Nevertheless, they acknowledge that in the absence of pathological signs, an NLD microbiota could be considered normal since *Lactobacillus*-deficient communities have been identified in the genital tract of otherwise healthy asymptomatic women [153]. This conception sets the stage for further research of the human microbiome, expands the possibilities to assess individualized receptivity based on the endometrial microbiome, and opens the door to explore targeted therapies for an altered endometrial microbial habitat.

3. Remarks

This review encloses different aspects of ER, spotlighting the opening of the WOI. Altogether, this compilation could aid in the development of new clinical practices that define an individual's receptive window for pET to improve ART results ultimately.

Nowadays, ER is the rate-limiting step in successful ART procedures that end up in pregnancy and child delivery. The endometrial tissue is a ponderous element in fertility; it constitutes the soil in which a viable embryo will implant to achieve progeny. The attainment of ER involves an extensive assortment of genetic and biochemical mechanisms that must integrate in a parallel manner. Understanding of this process as an entity is still insufficient; nevertheless, the surge of new technologies is contributing in the deciphering of receptivity mechanisms and in search of novel biomarkers that could serve to detect the WOI. Notably, although most studies focus on individual genetic mutations, a more comprehensive view of the parental genetics is needed to determine whether an endometrium is adequate for embryo transfer before the initiation of an ART procedure. Due to ambiguous or non-conclusive results in search of genetic predispositions of endometrial-associated infertility, it would be controversial to provide genetic counseling currently. Perhaps, in the future, massive sequencing could help to provide insights into the importance of single and multiple genetic mutations to establish a receptive or non-receptive profile. If assertive, this profile could be applied as an endometrial pre-implantation parental test to improve the rates of healthy pregnancies and live births. With this in mind, in our opinion, the best short-term approaches towards detecting or improving ER are the transcriptome, microbiome, and miRNA signatures, all achievable using the power of NGS. This review encloses different aspects of ER, spotlighting the opening of the WOI. Altogether, this compilation could aid in the development of new clinical practices that define an individual's receptive window for pET to improve ART results ultimately.

Abbreviations and nomenclatures

3D	three-dimensional
ApoE	apolipoprotein E
ART	assisted reproductive tech
CNTF	ciliary neurotrophic factor
CT1	cardiotrophin 1
E2	estrogen
ECM	extracellular matrix
EF	endometrial fluid
EGF	epithermal growth factor
EMX2	empty spiracles homolog 2
eNOS	endometrial nitric-oxide synthase
EnSCs	endometrial stromal cells
EPP	early-proliferative phase
ER	endometrial receptivity
ERA	endometrial receptivity array
ESP	early-secretory phase
ESR1	estrogen receptor 1
F13A1	coagulation factor XIII A chain
F2	coagulation factor II
F5	coagulation factor V
F8	coagulation factor 8
FAAH	fatty acid amide hydrolase
Fas L	Fas ligand
FGB	β fibrinogen
Foxp3	Forkhead box P3
gp130	glycoprotein 130
GPIIIa	glycoprotein IIIa
HAUSP	herpesvirus-associated ubiquitin-specific protease
HB-EGF	heparin-binding epithermal growth factor-like growth factor
hCG	human chorionic gonadotropin
HOXA10	homeobox A10
HOXA11	homeobox A11
HPA1	human platelet alloantigens 1
HSCORE	histological score
IGFBP1	insulin-like growth factor binding protein 1
IL11R	interleukin 11 receptor
IL6	interleukin 6
IP-10	IFN-γ-inducible 10 kDa protein
ITGB3	integrin β3
IVF	in-vitro fertilization
LD	lactobacillus-dominated
LH	luteinizing hormone
LIF	leukemia inhibitory factor
LIFR	leukemia inhibitory factor receptor
LPP	late-proliferative phase
LSP	late-secretory phase
MCP-1	monocyte chemo-attractant protein-1
MDM2	murine double minute 2
MHC	major histocompatibility complex
MiRNA	microRNA
MP	menstrual phase

MSCs	mesenchymal stem-like cells
MSP	mid-secretory phase
MTHFR	methylenetetrahydrofolate reductase
MUC1	Mucin 1
MUC4	Mucin 4
NGS	next generation sequencing
NLD	non-lactobacillus-dominated
OSM	oncostatin
P4	progesterone
TP53	tumor protein 53
PAI-1	plasminogen activator inhibitor-1
PCOS	polycystic ovarian syndrome
PCR	polymerase chain reaction
pET	personalized embryo transfer
PGE2	prostaglandin E2
PGF2α	prostaglandin F2α
PGs	prostaglandins
PIBF	progesterone-induced blocking factor
PP	proliferative phase
PR	progesterone receptor
PR-A	progesterone receptor A
RIF	repeated implantation failure
RPL	recurrent pregnancy loss
RSA	recurrent spontaneous abortion
SNP	single-nucleotide polymorphism
SP	secretory phase
TAM	tool annotations human miRNAs
TFF3	trefoil factor 3
Treg	T-regulatory cells
uNK	uterine natural killer
VEGF	vascular endothelial growth factor
VNTR	variable number tandem repeats
WOI	window of implantation

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Chapter 3

Aneuploidy Rates Inversely Correlate with Implantation during *In Vitro* Fertilization Procedures: In Favor of PGT

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Abstract

Aneuploidy, the hold of an abnormal number of chromosomes that differs from the normal karyotype, is a recognized leading cause of miscarriage and congenital disabilities. In human gametes and embryos, aneuploidy rates are prevalent, and these rates increase with advanced maternal age; additionally, it has been suggested that hormonal stimulation for achieving *in vitro* fertilization (IVF) protocols further increases aneuploidy rates. Although about 65% of chromosomally abnormal embryos culminate in spontaneous miscarriages, there is still evidence of live births harboring crucial aneuploidies. Furthermore, although some frequent aneuploidies are consistent, others differ between countries, making it harder to focus on a specific set of anomalies but vital to focus regionally on those more prevalent. Preimplantation genetic testing (PGT) is a highly endorsed technique in assisted reproductive treatments to evaluate possible embryo aneuploidies, genetic defects, and congenital disorders. On this subject, this study shows that IVF aneuploidy rates in embryo cohorts of high morphological quality are inversely associated with implantation rates. In its entirety, this study reinforces the utility of PGT for embryo evaluation.

Keywords: aneuploidy, preimplantation genetic testing, embryo implantation, *in vitro* fertilization, karyotype

1. Introduction

Aneuploidy is defined as a chromosome number that is not an exact multiple of the usually haploid number [1]. The terms haploid and diploid that describe single (n) and double (2n) chromosome sets in cells originate from the Greek terms *haplóos* meaning single and *diplóos* meaning double. The term ploidy was subsequently derived to describe the total chromosome content of cells. Consequently, the term euploid refers to a chromosome with an exact multiple of the haploid number [2]. Human body cells (somatic cells) are diploid, carrying two complete sets of chromosomes: one set of 23 chromosomes from their father and one set of 23 chromosomes from their mother; the two sets combined provide a full complement

of 46 chromosomes. Human gametes (or sex cells), sperm and oocytes, are haploid and contain only one set of 23 chromosomes.

Aneuploidies can occur either by chromosome gains (trisomies) and losses (monosomies) due to chromosome segregation errors, the so-called "whole chromosomal" aneuploidy or due to rearrangements of chromosomal parts, often accompanied by deletions, amplifications, or translocations of large regions of the genome that is referred to as a "structural" or "segmental" aneuploidy [3]. Whole chromosomal aneuploidies might arise due to random and sporadic chromosome missegregation events that occur with low frequency during any cell division. The missegregation levels range from 1/1000 to 1/10,000 in human cells [4].

Meiosis generates haploid gametes through a specialized cell division process that consists of one round of DNA replication followed by two cell divisions. The first division, or meiosis I (MI), involves the segregation of homologous chromosomes from each other, whereas meiosis II (MII) involves the segregation of the sister chromatids. Missegregation can also occur in germline cells, and the errors that arise in meiosis result in aneuploid embryos [5]. This chapter aims to provide evidence that supports the use of PGT for embryo evaluation and euploid embryo selection due to a positive correlation with fertilization rates.

2. Incidence of aneuploidy

Errors in meiotic chromosome segregation frequently occur during oogenesis ($\sim$20%), especially during the first meiotic division; this incidence of meiotic errors in oocytes is more elevated in women with advanced maternal age and may be due to the prolonged time that oocytes spend arrested at meiosis I stage, before ovulation [6]. However, some patterns of nondisjunction appear to be chromosome-specific; almost all cases of trisomy 16 are linked to errors at maternal MI, while MII errors are surprisingly common in trisomy 18. Oppositely, in sperm the incidence of aneuploidy is only 2%. Another considerable percentage of errors ($\sim$20%) arise during the first mitosis after fertilization. Among clinically recognized spontaneous abortions (fetal deaths occurring between 6 and 8 weeks and 20 weeks gestation), the incidence increases to $\sim$50% [7]; the most common specific abnormalities are sex-chromosome monosomy (45,X), accounting for nearly 10% of all spontaneous abortions, and trisomies 16, 21, and 22, which together constitute 50% of all trisomies identified in spontaneous abortions. The incidence among stillbirths (fetal

Chr	Case report	References
1	Pure duplication 1q41-qter: further delineation of trisomy 1q syndromes	[9]
	Partial duplication 1q: reports of five cases and review of the literature	[10, 11]
2	Duplication 2q2.1-q3.1	[12, 13];
	Partial trisomy 2q: two cases	[14]
3	Duplication 3p syndrome: reports of three cases and review of the literature	[15–17]
4	Patient with trisomy 4p	[18]
	Partial duplication and duplication region 4q28.3-qter in monozygotic twins with discordant phenotypes	[19, 20]
5	Trisomy 5p: reports of four cases report and review of the literature	[21–23]
6	De novo "pure" partial trisomy (6)(p22.3→pter): case report/review	[24]
	Familial trisomy 6p in mother and daughter	[25]
7	Interstitial de novo tandem duplication of 7 (q31.1-q35)	[26]
	New case of pure partial 7q duplication	[27]

Chr	Case report	References
8	Trisomy 8: report of four cases	[28]
9	Pure 9p trisomy derived from a terminal balanced unreciprocal translocation	[29]
	Trisomy 9: review and report of two new cases	[30]
10	Distal 10q trisomy with copy number gain in chromosome region 10q23.1–10q25.1	[31]
	Proximal 10q duplication in a child with severe central hypotonia	[32]
11	Partial 11q trisomy syndrome: two cases	[33, 34]
12	Clinical report of a patient with de novo trisomy 12q23.1q24.33	[35]
13	Trisomy 13, Patau's syndrome: reports of three cases	[36–38]
14	Partial proximal trisomy 14	[39]
15	Duplication of distal 15q: reports of 14 cases	[40, 41]
16	Partial trisomy/long arm of chromosome 16: case report/review of literature	[42]
	Complete trisomy 16: a case report	[43]
17	A 790 kb chromosome 17p13.3 microduplication: case report/literature review	[44, 45]
18	Trisomy 18, Edward's syndrome: reports of five cases and discussion	[46–48]
19	Three cases of trisomy 19	[49, 50]
20	20q11.2 duplication syndrome and pure trisomy 20p	[51, 52]
21	Cardiovascular and general health status of adults with trisomy 21	[53]
22	Trisomy 22 syndrome: a report of four cases in newborns and literature review	[54–56]
X	Fragile X syndrome: a case report/review of clinical and molecular diagnoses	[57, 58]
Y	Morphology and pathogenesis of 47,XYY/47,XY patients super male syndrome	[59]

Chr, chromosome number.

Table 1.
Case reports of live births with complete or partial chromosomal abnormalities (mosaic or multiple aberrations are not considered).

deaths occurring between ∼20 weeks gestation and term) is ∼4% with the types of abnormality being similar to those identified in newborns, and ∼0.3% of live-born are aneuploid with the most common abnormalities being trisomies 21, 18, and 13 and sex-chromosome trisomies 47,XXX, 47,XXY, and 47,XYY [5, 8].

2.1 Aneuploidies and live births

Although about 65% of chromosomally abnormal embryos culminate in spontaneous miscarriages, there is still evidence of live births harboring crucial aneuploidies. **Table 1** describes cases that are well documented.

3. Impact of aneuploidy on the efficiency of ART

Assisted reproduction is a solution in many of the growing cases of infertile couples worldwide. A high rate of embryos produced *in vitro* presents chromosomal aneuploidy (∼50%), and such aneuploid embryos have reduced the potential for achieving a viable pregnancy. Such abnormalities are recognized as the leading cause of implantation failure and spontaneous miscarriage [60]. Among conceptions that survive to term, aneuploidy is the leading genetic cause of developmental

disabilities and mental retardation [5]. **Table 2** describes data from different infertility centers predominantly showing that aneuploidy rates are similar.

The relatively high aneuploidy rate observed in human embryos after an IVF/ICSI cycle has been attributed to the technique itself since this prevalence seems to be lower in natural conceptions [61]. Many hypotheses have been proposed that may explain these findings: (1) controlled ovarian stimulation treatments, (2) factors related to the ICSI technique and (3) lab conditions as embryo culture.

3.1 Ovarian stimulation and the incidence of embryo aneuploidy

To increase the number of oocytes that can be retrieved for IVF, gonadotrophins are commonly used for superovulation in humans. Exogenous administration of gonadotrophins results in higher concentrations of steroids that may affect oocyte and embryo quality. Ovarian stimulation effects have been well characterized mainly in the murine model and have shown that aggressive stimulation leads to a poorer embryo development potential that could increase the chromosomal abnormality rate [79]. In humans, studies are scarce and less conclusive. A recent study in a population of young normovulatory women showed that a high ovarian response after controlled ovarian stimulation with moderate gonadotropin doses did not increase the embryo aneuploidy rate. Indeed, the higher the ovarian response, the more the euploid embryos obtained [80]; the remaining question is whether this can also be extrapolated to infertile patients with good ovarian reserve.

3.2 Intracytoplasmic sperm injection (ICSI) technique and the incidence of embryo aneuploidy

ICSI has become critical for the treatment of severe male infertility. The principal feature of ICSI is the direct injection of spermatozoa into an oocyte, which facilitates the production of fertilized embryos regardless of semen characteristics, such as sperm concentration and motility. However, the chromosomal integrity of ICSI zygotes is degraded compared to zygotes obtained from an *in vitro* fertilization [81, 82]. During the ICSI procedure, a sperm pretreatment is performed to mimic the conditions of natural fertilization and support the progression of fertilization effects. Studies on mouse models revealed that the chromosomal integrity of zygotes derived from ICSI without any pretreatment of spermatozoa was impaired in comparison with zygotes derived from conventional IVF [83]; even the culture sperm conditions may affect the chromosomal stability of the embryo [84]. Chromosomal damage may occur due to the injection of non-capacitated, acrosome-intact spermatozoa, so to reduce the risk of chromosomal aberrations during the ICSI procedure, it is crucial that sperm capacitation and the acrosome reaction be appropriately artificially induced in the proper medium before use [85].

3.3 Embryo culture and the incidence of embryo aneuploidy

Fertilization and embryo development *in vitro* have the potential to introduce (often inadvertently) stress which cannot only impair embryo development in the laboratory but also have downstream effects after transfer.

In vivo, the developing preimplantation embryo is exposed to gradients of nutrients, hormones, cytokines, and growth factors as it progresses through the fallopian tube to the uterus. Within the lumen of the female tract, the embryo resides in a few 100 nanoliters of a complex viscous fluid characterized by high levels of mucins, albumin, and glycosaminoglycans and by reduced levels of oxygen

N samples (country)	Day of biopsy	Aneuploidy rate	Trisomy %	Monosomy %	Most affected chromosomes	Less affected chromosomes	Ref.
87 (India)	3	54	14.9[*]	42.5[*]	22, 18	No data	[62]
150 (Japan)	5	40.6	18[*]	21.3[*]	15, 22, 21, 16, 18	4, 12	[63]
52 (UK)	5	40.4	51.3[#]	48.7[#]	22, 16, 15, 18, 21, X	1, 2, 5, 10, 17, 19	[64]
12 (UK)	3	75	22[*]	11[*]	20, 21, 22	6	[65]
5879 (USA)	3	70.6	No data	No data	No data	No data	[66]
	5	47.8					
759 (UK)	3	64.6	40[#]	60[#]	16, 22, 21, 4, 5	4, 6	[67]
274 (US)	3	72.3	39.8[*]	44.5[*]	22, 16, 7	6, 9, 19	[68]
192 (Italy)	5	55.2	37.9[*]	42.7[*]	No data	No data	[69]
240 (Mexico, Center A)	5	36.3	5.34[*]	~5[*]	16, 22, XXX, 9	3, 7, 8, 10, 12, 18, 20	[70]
	6	61.1	5.55[*]	~10[*]			
210 (Mexico, Center B)	5	48.9	15.96[*]	~4[*]	15, 16, 21, 4	1, 2, 3, 5, 8, 9, 10, 11, 12, 14, 17, 20, 22, X, Y	
	6	43.1	14.65[*]	~5[*]			
404 (Mexico)	No data	60.89	No data	No data	4, 15, 22, 16	No data	[71]
15,169 (USA)	No data	No data	No data	No data	13, 15, 16, 18, 19, 21, 22	1, 12, 3	[72]
2204 (UK)	0	74	56[#]	44[#]	16, 21, 22, 15, 19	No data	[73]
	3	83	49[#]	51[#]	22, 16, 19, 21, 13		
	5	58	47[#]	53[#]	22, 16, 15, 21, 19		
21 sets (Italy)	0/1	97.4	Chromosomal loss three times more frequent than gain		22, 15, 16, 17	No data	[74]
	3	47.6[+]					
	5 or 6	80[++]					
195 (USA)	0/1	65.5	39.86[#]	60.14[#]	22, 13, 15, 16, 19, 21	6, 5, Y, 3	[75]
	5/6	45.2	52[#]	48[#]	22, X, 16, 18, 21		
1025 (Mexico)	3/5	43.9	59.3[#]	40.7[#]	16, 21, 22, 19, 15, 20	8, 4, 3, 2, 7, 1	Current study

[*]*Percentage of the total number of samples.*
[#]*Percentage of the total number of aneuploid samples.*
[+]*Rate from the previous stage of development, PBs to blastomere.*
[++]*Rate from the previous stage of development blastomere to TE, PBs = polar bodies, TE = trophectoderm.*
For the current study, infertile patients who underwent ART at the Ingenes Institute were included. The patients were clinically evaluated according to a standardized protocol that includes family and personal clinical history. The protocol was approved by the Ethics Committee of the Ingenes Institute, and a signed informed consent was obtained from all patients. IVF, embryo biopsy, and mCGH were performed according to the standard protocols of the Institute Ingenes as previously described [76, 77]. Only optimal morphological embryos were considered for this study. Selection and embryo transfer were done on Day 3 or Day 5 of development according to the embryo morphological assessment, using the criteria established by the Istanbul consensus Workshop on Embryo Assessment [78].

Table 2.
Aneuploidy rates of different IVF clinics around the world; when mentioned, the most commonly affected chromosomes are listed.

(typically 2–8%). The embryo is in constant motion, moved by gentle ciliated and muscular action of the female tract [86]. This scenario is in stark contrast to the laboratory environment, where typical gametes and embryos are exposed to relatively large volumes of culture medium, remain static during culture while resting on a polystyrene substrate, and create unstirred layers where the end products of metabolism concentrate and nutrients become limited [87].

Embryos are sensitive to both chemical and physical signals within their microenvironment. Factors within the laboratory as oxygen level, ammonium released from amino acids into the culture, poor laboratory air quality, temperature and pH, oil overlay, embryo culture volume/density, the static nature of culture, light, or even mechanical factors as pipetting, can negatively impact gametes and embryos and generate stress. When more than one stress factor is present in the laboratory, more negative synergies can result, and these factors play a significant role in influencing the development and events post transfer [88]. For example, recent studies have reported that a decrease in temperature has the potential to affect the stability of the oocyte's meiotic spindle, reducing fertilization rates, delaying embryo development, and decreasing clinical pregnancy rates [89]. However, more studies are needed to demonstrate the impact of embryo culture on aneuploidy rates.

4. Aneuploidy detection: techniques for PGT

PGT is the genetic diagnosis analysis performed to identify euploid embryos before uterine transfer [90]. PGT determines the numeric chromosomal constitution of a cellular biopsy sample obtained from a cultured embryo to determine its competence [91, 92].

PGT was first described in 1990 by Handyside et al. [93] when the sex of the six- to eight-cell stage embryos from two couples with a known risk of transmitting X-linked diseases was assessed by DNA amplification of a Y chromosome-specific repeat sequence. The earliest PGT studies in the 2000s were based on the fluorescence in situ hybridization (FISH) technique where 3–12 chromosomes can be analyzed on the cleavage stage or polar body biopsies [90]. Those studies had disappointing results in clinical practice since it had no beneficial effect on live birth rate after IVF [94]. The major drawback of FISH-based PGT is the limited number of chromosomes that can be analyzed considering that aneuploidy can affect any of the 22 autosomes and both sex chromosomes [95]; consequently, there have been dramatic improvements in PGT technology to make it valuable for clinical practice.

Nowadays, several methodologies for 24-chromosome analysis are available for clinical use that aim to increase implantation rates and decrease miscarriage rates associated with IVF [90]: microarray comparative genomic hybridization (mCGH), single-nucleotide polymorphism (SNP) microarray, real-time polymerase chain reaction (qPCR), and next-generation sequencing (NGS) [96, 97]. This review will focus on the relevant aspects of the PGT techniques used in our laboratory.

4.1 Microarray comparative genomic hybridization (mCGH)

mCGH is a ratio labeling protocol to compare the DNA product of a clinical sample to a healthy control. For PGT, biopsied embryonic cells must be lysed to extract the sample's DNA, which will be further amplified by a protocol that provides whole genome coverage [90, 95, 98]. The resulting DNA products are co-hybridized with a standard DNA control sample (46,XY and 46,XX) with a series of site-specific fluorophores on a microarray chip with approximately 4000 markers spaced throughout the genome [90]. Then, a confocal laser platform

detects the relative color intensity, and a bioinformatics compares the intensity of each fluorophore in the sample versus the control to identify any bias and determine the ploidy status of the sample [90, 95, 98].

The mCGH analysis reports the ratio of sample DNA to a reference DNA, as a chromosomic profile where the molecular karyotype is represented. Usually, the sample DNA is labeled with a green fluorescent dye, while the reference DNA sample is tagged red [99]. Thus, diploid embryos will have a relatively equal ratio of green-to-red fluorescence in every pair of chromosomes, represented as a continuous horizontal plot line. Monosomy will be represented as a clear downward deviation in the plotted line, indicating a relative lack of green-to-red signal intensity; in contrary, a trisomy will be displayed as an upward deviation in the plotted line due to a relative increase in the green-to-red signal intensity.

The specificity rate of mCGH-based PGT is about 99% [90]. The test results can be available within 12–15 h, considering that the entire analysis can be performed during this short time frame [90, 91]. Additionally, brand-specific features are offered by each manufacturer: Agilent's GenetiSure Pre-Screen Microarray offers a detection rate of 100% for aberrations >10 Mb and 89% for >5.3 Mb [100]; KaryoLite BoBs Kit from Perkin Elmer uses an alternative BACs-on-Beads technology and results are interpreted by the BoBsoft™ analysis software [99]; and RHS's EmbryoCellect Kit is the only mCGH-based PGT validated for mosaicism detection [101]. Recently, Illumina's 24sure PGS Microarray had been discontinued, and the NGS-based VeriSeq PGS is now offered as an alternative solution [102].

mCGH entails some disadvantages: first, the embryo sample requires a previous whole genome amplification (WGA) process to support single-cell diagnostics by mCGH [95], raising the possibility of introducing errors during the amplification [91]; second, mCGH is a semiquantitative technique that only reports the ratio of sample DNA to a reference DNA; it is to say that only imbalances in DNA content can be identified. Therefore, mCGH is unable to detect uniparental disomic or triploid embryos as it cannot discriminate between 46,XX from 69,XXX, and 46, XY from 69,XXY [90, 91, 95]. Last, the mCGH used for PGS cannot identify structural chromosome aberrations or diagnose mosaicism in a trophectoderm sample [90].

4.2 Next-generation sequencing (NGS)

NGS refers to the emerging technology of non-Sanger-based DNA sequencing that allows the sequence in parallel millions of DNA strands with high-throughput yield. In the field of ART, this powerful tool is being applied for PGT to replace cytogenetic microarrays [98, 102].

Different platforms are commercially available for NGS with different technological approaches. Illumina's MiSeq NGS platform applies a sequencing-by-synthesis method, where DNA is attached and amplified in situ to be subsequently used as a template for synthetic sequencing with fluorescent-labeled reversible-terminator nucleotides [103]. Ion Torrent NGS technology, commercialized by ThermoFisher Scientific, is based on collecting data by sensing the hydrogen ions that are released as by-products when nucleotides are incorporated by a template-directed DNA polymerase synthesis on an ion chip [104].

Despite the dissimilarities between platforms, the common basis of chromosome copy number analysis by NGS is the fragmentation of the amplified DNA sample into small segments of 100–200 base pairs that are further sequenced in parallel until the number of reads covering a determined position in the genome is attained, in general, a 30× coverage (sequencing each base pair 30 times) ensures sufficient accuracy. The sequence data obtained are then compared with a reference genome

and counted by bioinformatics software. The copy number of a specific chromosome should be proportional to the number of counted sequences; therefore, an increase or reduction in the number of reads will, respectively, represent a trisomy or monosomy [97, 99].

NGS allows to simultaneously perform both qualitative and quantitative analyses of multiple embryos with high-resolution data for chromosomal analysis [96, 97]. The higher sensitivity and precision offered by NGS [96, 105, 106] makes possible to exclude embryos with mosaicism [105, 106] and partial aneuploidies or triploidies [106], improving pregnancy outcomes due to its enhanced capability for detecting those challenging abnormalities.

PGT by NGS can predict not only chromosome copy number for the diagnosis of whole chromosome aneuploidy with 99.98% assignment consistency [97] but also single-gene disorders [107], abnormalities of the mitochondrial genome [108], and segmental chromosome imbalances [97, 99]. Balanced chromosomal rearrangements cannot be detected by NGS [97].

The increasing demand and accelerated development are continuously reducing the cost of NGS technology [109]. Also, potential cost-benefit ratios can be achieved when the full sequencing capacity of the apparatus is exploited [96, 97, 99]. Furthermore, molecular tools, like barcoding, are being implemented to allow multiplex high-throughput sequencing [110]; this promising strategy will reduce the diagnosis' cost per patient by performing simultaneous analysis of multiple embryos from different patients [97].

5. Aneuploidy and women age

In our study, by analyzing the mCGH data, the total number of aneuploidies was found to be 734, and from these, 641 (87.3%) were derived from patients and 93 (12.7%) from donors. Overall, this study displayed similar rates of monosomies, trisomies, double aneuploidies, and multiple aneuploidies. The total number of monosomies (191) was similar to the number of trisomies (194), accounting for 26 and 26.4% of the total aneuploidies, correspondingly. Furthermore, the total number of double (165) and multiple (184) aneuploidies was also very similar, accounting for 22.5 and 25.1% of the total aneuploidies, correspondingly. Nevertheless, it is worth noticing that when considering only the donor group, monosomies seem to be more prevalent: 38.7% of the total donors' aneuploidies were monosomies vs. 24.7% of trisomies, 16.1% of double aneuploidies, and 20.4% of multiple aneuploidies; what is more, the percentage of monosomies in the donor group is higher than that of the monosomies of the patient group (38.7 vs. 24.3%). The most common monosomies affected chromosomes 15, 16, and 22, whereas the most common trisomy affected chromosomes 16, 19, and 21 (**Table 3**).

It has been shown that the lowest risk for embryonic aneuploidy is between ages 26 and 30, with aneuploidy rates steadily increasing with maternal age after 26 years of age [111] and leaping significantly from the age of 39 [112]. For this reason, women of advanced maternal age are encouraged to favor oocyte donation to yield high-quality viable embryos.

Interestingly, some studies have identified that women of younger ages possess an increased prevalence of aneuploidy, with >40% of aneuploidy in women of 23 years and under [111] and 58% of aneuploidy in women of <31 years of age. In the current study, both the donor (≤29 years) and the patient group of ≤29 years

	All	Patients	Donors
Total	734	641	93
Monosomy	191 (26.0%)	155 (24.3%)	36 (38.7%)
−Chr 15	13	13	0
−Chr 16	21	18	3
−Chr 22	22	22	0
−Chr X	10	8	2
−Chr Y	40	26	14
Trisomy	194 (26.4%)	171 (26.7%)	23 (24.7%)
+Chr 16	26	24	2
+Chr 18	9	7	2
+Chr 19	23	21	2
+Chr 20	17	13	4
+Chr 21	20	20	0
+Chr 22	19	19	0
+Chr X	10	8	2
+Chr Y	1	1	0
Dual	165 (22.5%)	150 (23.4%)	15 (16.1%)
Multiple	184 (25.1%)[a]	165 (25.7%)[b]	19 (20.4%)[c]

The current study included 441 patients, resulting in 474 cycles. A total of 1629 embryos were analyzed; from those, 54 were excluded due to failed WGA, leaving 1575 embryos for analysis, 1258 from patients, and 317 from donors. Biopsies were performed at the blastomere (Day 3, patients = 238 and donors = 50) and blastocyst stages (Day 5, patients = 1020 and donors = 267). Finally, 734 embryos (46.6%) were found to be aneuploid (patients = 641 and donors = 93). The total number of monosomies and trisomies is provided along with their respective percentages; furthermore, the number of the most common aneuploidies of the mCGH data is listed.
[a]*Nine embryos had completely abnormal mCGH profiles.*
[b]*Even embryos had completely abnormal mCGH profiles.*
[c]*Two embryos had completely abnormal mCGH profiles.*

Table 3.
Most frequent types of aneuploidies in the mCGH data of the current study.

displayed high aneuploid rates, 28.5 and 27.4%, respectively (**Table 4**). Given the high rates of aneuploidy in younger women, attention should be paid in detecting aneuploidy in embryos from women of young maternal age, especially since this group of patients is not routinely encouraged to perform a PGT. Still, whether there is a difference between the distribution of aneuploidies between donors and patients remains uncertain.

When stratifying our analysis in age groups (a, ≤29; b, 30–34; c, 35–37; d, 38–40; e, 41–43; and f, ≥44 years of age), a visible continuous increase in aneuploidy rate can be observed as maternal age increases (**Table 4**); furthermore, this increase in aneuploidy goes hand in hand with a continuous decrease in implantation, as it can be observed in the decrease of positive beta-human chorionic gonadotropin (β-hCG) values as age increases (β-hCG values ≥10 mUI/ml from Day 14 after transference were considered positive).

Category	≤29 a	30–34 b	35–37 c	38–40 d	41–43 e	≥44 f	Donors g
Number of samples (n, cycles)	27	66	72	105	90	31	83
Age (years)	27.0 ± 2.4[b,c,d,e,f,g]	32.5 ± 1.3[a,c,d,e,f,g]	36.1 ± 0.7[a,b,d,e,f,g]	39.0 ± 0.8[a,b,c,e,f,g]	41.8 ± 0.8[a,b,c,d,f,g]	45.0 ± 1.6[a,b,c,d,e,g]	22.8 ± 3.0[a,b,c,d,e,f]
Body mass index (kg/m²)	23.6 ± 3.8[g]	24.6 ± 3.8[g]	24.3 ± 3.9[g]	24.4 ± 3.7[g]	24.8 ± 3.8[g]	24.6 ± 2.8[g]	21.8 ± 2.5[a,b,c,d,e,f]
Ova collected (n)	16.8 ± 7.9[e,f]	17.3 ± 9.8[d,e,f]	16.1 ± 9.0[d,e,f]	12.0 ± 6.3[b,c,g]	11.0 ± 5.9[a,b,c,g]	8.9 ± 6.0[a,b,c,g]	15.8 ± 8.1[d,e,f]
Ova fertilized (n)	14.3 ± 6.9[e,f]	15.2 ± 8.6[d,e,f]	13.6 ± 7.5[d,e,f]	10.3 ± 5.2[b,c,g]	9.8 ± 5.5[a,b,c,g]	7.6 ± 4.8[a,b,c,g]	13.9 ± 6.2[d,e,f]
Embryos (n)	11.2 ± 6.3[e,f]	11.7 ± 7.9[d,e,f]	10.6 ± 6.0[d,e,f]	7.7 ± 4.2[b,c,g]	7.5 ± 4.6[a,b,c,g]	6.2 ± 3.9[a,b,c,g]	10.4 ± 4.6[d,e,f]
Fertilization rate (%)	77.9 ± 17.0	76.3 ± 15.1	79.7 ± 14.2	76.6 ± 16.6	77.0 ± 18.9	83.6 ± 15.2	76.1 ± 16.0
Aneuploidy rate (%)	27.4 ± 33.3[d,e,f]	36.0 ± 29.2[d,e,f]	35.7 ± 30.3[d,e,f]	57.7 ± 34.9[a,b,c,f,g]	66.6 ± 37.7[a,b,c,g]	87.4 ± 19.5[a,b,c,d,g]	28.5 ± 28.7[d,e,f]
Pregnancy rate (%)	60.87[b,c,d,e,f,g]	50.99[a,c,d,e,f,g]	49.09[a,b,d,e,f,g]	44.00[a,b,c,e,f,g]	55.17[a,b,c,d,f,g]	42.86[a,b,c,d,e,g]	50.79[a,b,c,d,e,f]

Values are shown as mean ± standard error. Significance was determined by one-way ANOVA followed by a Bonferroni or Dunnett's T3 post hoc test. Superscripts indicate a significant difference (p < 0.05, two-tailed):

[a]Versus ≤29 years old group.
[b]Versus 30–34 years old group.
[c]Versus 35–37 years old group.
[d]Versus 38–40 years old group.
[e]Versus 41–43 years old group.
[f]Versus ≥44 years old group.
[g]Versus donors.

Table 4.
Comparative of aneuploidy and pregnancy rates between age groups.

6. Remarks

One of the most critical reasons for unsuccessful IVF procedures is implantation failure due to aneuploid embryos. Aneuploidies are the primary cause of perinatal death and genetic abnormalities; consequently, the detection of chromosomal disorders constitutes the most frequent indication for PGT. Here, we report on the aneuploidy rates found in IVF procedures in Mexico. Even though there are studies that assert that PGT does not improve pregnancy rates, we show that aneuploidy rates inversely correlate with implantation and that levels of aneuploidy among high morphological quality embryos are still an important issue to be faced in everyday ART practice, and this evidence works in favor of continuing to use PGT analysis.

Acknowledgements

Abbreviations

ANOVA	analysis of variance
ART	assisted reproductive techniques
Chr	chromosome
FISH	fluorescence in situ hybridization
ICSI	intracytoplasmic sperm injection
IVF	*in vitro* fertilization
mCGH	microarray comparative genomic hybridization
MI	meiosis I
MII	meiosis II
NGS	next-generation sequencing
OR	odds ratio
PGS	preimplantation genetic screening
PGT	preimplantation genetic testing
qPCR	real-time polymerase chain reaction
SNP	single-nucleotide polymorphism
WGA	whole genome amplification
β-hCG	beta-human chorionic gonadotropin

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Screening (Bi Test, Triple Test, Panorama Test) and Amniocentesis for Early Diagnosis of Congenital Malformations

Gladys Cristina Al Jashi and Isam Al Jashi

Abstract

The genetic consult is very important in the diagnosis of early fetal malformations and its complications at birth and after it. Our research is based on a 3-year research on 6097 pregnant women who underwent screening *Bi-Test or Triple Test*. We discovered 408 pregnant women who were found positive and needed amniocentesis for a diagnostic of certitude. Out of them, 14 had a positive result from which 10 were found with Down syndrome and 4 with Edwards syndrome. In Romania, amniocentesis has become the most used method of prenatal diagnosis for pregnant women at 35 or above with a family history of hereditary congenital anomalies. However, the latest screening test from maternal blood, the Panorama test, can discover many malformations (for chromosomes 21, 18, and 13 and the abnormality of the sex chromosome). The accuracy for false positive is 2% and false negative 98%. In that light, the purpose of our study is to decrease the use of amniocentesis and to introduce the latest tests (Panoramic) for the early diagnosis of fetal malformation, the use of maternal blood, and the avoidance of using invasive medical procedures.

Keywords: screening *Bi-Test*, *Triple Test*, *Panorama Test*, amniocentesis for early detection of fetal malformations

1. Introduction

Talking with the patient about his or her family medical history, such as if the couple had a person with Down syndrome in their family or any other malformation, is important. That can be considered as a 'genetic consultation' in order to find out about any possible genetic problems before screening or any other tests. The specialist tries, in the first place, to make the patient understand the nature of his disease he is confronting, what's the most possible evolution of it, and last but not least what are the possibilities of treating it. A major objective of the genetic consult is to make the patient understand what are the factors that lead to the development of the disease, what are the mechanisms that transmit the disease, and how high is the risk of appearance to other family members. The doctor establishes, after running different tests, the correct diagnosis of the disease, its evolution, its prognosis, as well as the possibilities of treatment for a higher quality of life.

The genetic consult is very important in the diagnosis of early fetal malformations and their complications at birth and after it. The consult is necessary for pregnant women with high risk in developing a malformation (e.g., if the woman has in the family history a newborn with malformations, if she gave birth before to a baby with malformations, if she lives in a toxic environment).

In Romania, it is mandatory that every pregnant woman should do the BI-TEST or TRIPLE TEST, depending on the pregnancy weeks.

The cytogenetic prenatal diagnosis refers to early detection of intrauterine fetal abnormalities and genetic malformations, very often determined by genetics, which complicate and threaten the life of 3–5% of newborn. These abnormalities explain the 20% deaths in the newborn period and the much higher percentage of deaths in childhood and puberty [1]. From the fetal abnormalities, 95% are unexpected and appear at pregnancies that are not considered at risk for these situations. They can be light or severe.

The severe abnormalities are not compatible with life or can cause a long-term handicap [2].

There are multiple purposes for the detection of intrauterine prenatal illness such as:

- Establishing the high-risk diagnosis and possibly the termination of the pregnancy (abortion), the pregnancy representing a high risk of death both for the mother and fetus

- Identifying a situation that can influence the date, the place, or the childbirth method

- Identifying fetuses that will benefit of early pediatric intervention

- Identifying situations that can affect future pregnancies

Opinions are different when it comes to knowing the diagnosis; many families decide to keep the pregnancy even if there are severe malformations, thinking that they can fight this problem by taking care of the baby and not terminating the pregnancy. But most of the families decide to terminate the pregnancies and seek treatments for future pregnancies.

In conclusion, the most important thing in the prenatal diagnosis is obtaining very early in the pregnancy of correct informations about the fetus and making a correct and easy decision in conformity with family principles [3].

The indications for prenatal diagnosis are:

- Pregnancy—women at age of 35 or above

- Husband aged 45 or above

- Past pregnancy with a fetus with chromosomal abnormality

- One of the parents has a chromosomal abnormality including being a carrier of a balanced translocation or other structural rearrangements

- Down syndrome

- Pregnancies with risk of Mendelian or polygenic diseases (e.g., Tay-Sachs disease)

- Abnormal fetus diagnosed by the ultrasound procedure within the first weeks of pregnancy

- Maternal anxiety

- Determination of fetal risk in pregnancies with high risk for hereditary x-linked diseases, for that there is not yet a specific prenatal diagnosis (it is obtained better by determining the Y-chromosomal material with the new ADN techniques)

- History of two or more abortions without the possibility of effectuation of the cytogenic analysis to both parents or a noninvasive prenatal diagnosis [4]

The screening programs focus on the most common chromosomal abnormality—the Down syndrome (trisomy 21)—and they not only detect very often other fetal abnormalities.

2. The BI-TEST

The screening of the first trimester is a prenatal text that offers informations about early pregnancy and the risk of the baby to develop some specific chromosomal abnormalities—the Down syndrome (trisomy 21) and the Edwards syndrome (trisomy 18).

The Down syndrome determines a lifetime affectation of the mental and social development and also some physical modifications. As for Edwards syndrome, it is a much more severe condition, and often it is fatal until the early age of 1 year [5].

The screening tests from the first trimester of pregnancy do not evaluate the risk of defects of the neural tube, such as spina bifida.

We do the BI-TEST (the screening of the first trimester in pregnancy) because it can be done much earlier than other tests of screening; the results can be available in the first weeks of pregnancy, which offers the parents much more time in taking the best decision regarding additional tests, medical treatment, and the course of pregnancy [6].

Normally, the screening of the first trimester (the BI-TEST) is made between weeks 11 and 14 of pregnancy. The results of the test are formulated as positive or negative and also like a probability, e.g., 1:5000 risk to have a baby with Down syndrome. In general, the test is considered positive if the risk is 1:300 or above. The BI-TEST implies two steps: a blood test that measures the level of two hormones that are specific in pregnancy (pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG)) and an ultrasound examination which measures the size of the transparent space behind the neck of the fetus (the nuchal translucency) [7]. Correlating the values obtained from the blood tests with the ultrasound examination and some specific parameters of the mother (age, height, weight, blood pressure, smoking, diabetes, assisted reproductive techniques), the specialist can calculate the risk of the mother of having a baby with chromosomal abnormalities [6].

Frequently, a positive result will enforce the performance of secondary diagnosis tests:

- The chorial biopsy—it is done normally in the first trimester of pregnancy, and it is an invasive test which implies cropping a piece of the placenta, with a low

risk of abortion. It is used to detect chromosomal abnormalities, like the Down syndrome.

- Amniocentesis—it can be used to identify chromosomal abnormalities but also defects of the neural tube, like spina bifida. During the amniocentesis, which frequently is done in the second trimester of pregnancy, a small amount of amniotic liquid is estranged. It also had a low risk of abortion [6].

3. The TRIPLE TEST

A continuance of the BI-TEST from the first trimester and the screening TRIPLE TEST (made in the second trimester of pregnancy) is more and more often requested by us. With the help of it, we can evaluate the risks of the baby developing a neural tube disorder (spina bifida) or chromosomal abnormalities such as the Down syndrome (trisomy 21) or the Edwards syndrome (trisomy 18) [6].

Between the 15 and 20 weeks of pregnancy (with optimal results in the weeks 16–18), pregnant women can be tested with a simple blood test known as the TRIPLE TEST (or triple marker test or second trimester screening). It is made in combination with an ultrasound examination [3].

For the triple marker testing, a blood test is required that will measure the levels of:

- hCG (human chorionic gonadotropin) secreted by the placenta.

- Unconjugated estriol, a product of the placenta and the fetus.

- Alpha-fetoprotein (AFP gene) produced by the fetus's liver.

- Sometimes it also tested the inhibin A (produced by the placenta), because it considerably grows the chances of detection of the Down syndrome, which is also known as the quadruple test.

The level of each substance present in the blood helps the doctor identify if the fetus is exposed to the risk of developing congenital disorders and chromosomal abnormalities. Exactly as in the case of the BI-TEST, multiples of the median (MoM) is measured depending on the clinical data of the mother (age, height, weight, race, smoker, age of the fetus) [4]. The results of the tests are available in 2 weeks from the blood tests. Usually, if the results of the TRIPLE TEST show a high level of alpha-fetoprotein, it indicates a neural tube defect. The doctor will recommend an ultrasound examination of the fetal cranium and spinal cord. The mother is also purposed to do an amniocentesis, which involves extracting a small amount of amniotic liquid from the uterus for further investigation.

On the other hand, low levels of alpha-fetoprotein (AFP) and unconjugated estriol indicate a high risk of Down syndrome. In this situation, it is recommended that an amniocentesis should be done.

4. Amniocentesis

Amniocentesis is a medical procedure used in prenatal diagnosis of chromosomal abnormalities, in which a small amount of amniotic fluid which contains fetal tissues is sampled from the amniotic sac and which is afterward tested.

It is a two-person procedure which requires a lot of experience and care from the side of the doctor. One of the doctors assures the ultrasound examination, and the

other doctor places the puncture needle. This way, he can manipulate the needle with all the attention and care to attain the desired target. There has to be an incredible collaboration between both doctors, because they have to anticipate each other's moves.

Before the procedure, it is mandatory that the sanguine groups ABO and RH be determined and also to perform an ultrasound examination, which will determine the number of fetuses, the position and viability, the localization of the placenta, the amount of amniotic fluid, and the largest amniotic sac that will be punctured and will assure that the fetus is anatomically normal. Also the gestational age of the fetus by the fetal biparietal diameter, the length of the thigh bone, and the abdominal circumference is determined.

Choosing the place of puncture is made after the initial evaluation. When doing the amniocentesis, it is important to choose the place of the puncture needle in the amniotic cavity wisely, namely by carefully avoiding the placenta, the umbilical cord insertion place and the fetus. Colored Doppler ultrasound can facilitate the choosing of the place for the transplacental puncture, to avoid fetal hemorrhage.

Diagnosis of chromosomal abnormalities and diagnosis of the neural tube defects are the most common indications for amniocentesis in the beginning of the second trimester of pregnancy. As for the evaluation of the fetal pulmonary maturation, it is the most common indication for amniocentesis in the third trimester [8].

At the end of the first trimester and the beginning of the second trimester, the indications for amniocentesis are:

• Cytogenetic diagnosis

• Diagnosis of neural tube defects

• Diagnosis of metabolic disorders

At the end of the second trimester and the beginning of the third trimester, the indications for amniocentesis are:

• Evaluation of the severity of Rh immunization

• Evaluation of fetal pulmonary maturation

• Diagnosis of intra-amniotic infections

• Confirmation of the ruptured membranes

Last, but not the least, the therapeutic purpose of the amniocentesis has indications in the polyhydramnios drainage and the medical treatment of fetal diseases.

Ultrasound examination (localization of the placenta, fetus, and amniotic liquid bag) and the insertion of the needle can take up to 20 min. Extraction of the amniotic liquid can take up to 5 min.

Some women don't feel pain (painless procedure), while other pregnant women feel cramps as the needle punctures the uterus or a pressure sensation during the procedure of extracting amniotic liquid.

Local anesthetic is not normally used. Associated factors to the high level of pain during the amniocentesis are considered to be maternal anxiety, presence of menstrual cramps in the past, an anterior amniocentesis, and the insertion of the needle in the inferior part of the uterus, where the pain is less felt by the patient [9], although there aren't any data to support this practice [10].

5. Panoramic Test

The Panorama Test is a prenatal noninvasive test, which analyzes the fetal DNA from the maternal blood and establishes the risk of common fetal aneuploidies in pregnant women, most interestingly the 21, 18, and 13 chromosomes but also the analyzes the sexual chromosomes X and Y.

Aneuploidies are chromosomal abnormalities characterized by the alteration of the number of chromosomes, for example, more or less chromosomes instead of the exact number of chromosomes; the trisomy, when there is an extra chromosome; or monosomy, when there is missing a chromosome.

Trisomy 21 is caused by the presence of an extra chromosome to the 21st pair, and it's known as the Down syndrome. It is the most frequent cause of mental retardation. The patients with Down syndrome present cardiac disease or other diseases which require surgical interventions or specific medical treatment. Other symptoms are, for example, ophthalmological problems [11].

Trisomy 18 is caused by the presence of an extra chromosome to the 18th pair, and it's known as the Edwards syndrome. It is a serious cause of mental retardation. The majority of the children with trisomy 18 present severe cerebral malformations, severe heart malformations, or severe malformations of other organs. Often, the growth retardation is found, which can lead to the termination of pregnancy (abortion). Most of the children who are born with the growth retardation die within their first year of life. Patients who survive present severe intellectual disability and have problems of growth and development [12].

Trisomy 13 is caused by the presence of an extra chromosome to the 13th pair, and it's known as the Patau syndrome. It is a cause of severe mental retardation. The majority of the patients present serious cerebral and other organs abnormalities. In many situations the pregnancy doesn't get to its due date, and the newborn dies very fast. Half of the born babies die in their first month of life, and 90% from those who survive in the first year of life present heart malformations, renal malformations, and renal malformations.

Aneuploidies of the sexual chromosomes are caused by the presence or the absence of one of the sexual chromosomes. The test can detect the risk for XXX, XYY, XXYY, and XXY (the Klinefelter Syndrome) and for the X monosomy (the Turner syndrome). A significant variability exists in regarding the severity of these genetic conditions, but the majority of the affected individuals are characterized by slight modifications physically and mentally.

During pregnancy some fragments of the fetal DNA run through the maternal blood. The fetal DNA is detectable since the 5th week of pregnancy. The concentration increases successively in the next weeks of pregnancy and disappears right after childbirth. The circulating fetal DNA quantity is found from the 9th to 10th weeks of pregnancy, and it's sufficient to guarantee the relevance and the sensibility of the Panorama Test.

The test is done by collecting a blood sample from the pregnant woman from at least the 10th week of unique and twin pregnancies. In the laboratory a complex analysis is made, where the fetal DNA from the maternal blood is isolated. This procedure identifies the eventual chromosomal abnormalities, like aneuploidy, and cutting-edge sequencing technology is used, along with advanced bioinformatics analysis.

The results obtained in the Panorama Test are:

- "High risk" implies that there is a probability that the fetus can present an aneuploidy of the 21st, 18th, and 13th pairs of chromosomes or an aneuploidy of

X and Y sexual chromosomes. This result indicates that there is a high chance that the fetus can present one of the abovementioned abnormalities, but it is not sure the fetus will have that condition. The endorsed advice is an invasive prenatal diagnosis test, like chorionic villus biopsy or amniocentesis.

• "Low risk" implies that the test detected a risk <1/10,000 (0.01%) for the fetus to be affected by a chromosomal aneuploidy.

• In some cases, when the quantity of fetal DNA is not enough, the test can show.

• Incomplete results or no results. In this situation, a new blood sample will be collected for the repetition of the test. In case the sexual analysis is demanded, this result can also be provided.

The accuracy of the Panorama Test

	% diagnosis (sensibility)	% false positive (specificity)
Trisomy 21 (the Down syndrome)	99 (>99%)	<0.1% (>99%)
Trisomy 18 (the Edwards syndrome)	99 (>99%)	<0.1% (>99%)
Trisomy 13 (the Patau syndrome)	08/10/16	<0.1% (>99%)
Determination of the fetal sex	99 (>99%)	<0.1% (>99%)

The test can detect the risk of a sexual chromosomal aneuploidy with a rate of detection which varies depending on the type of detected aneuploidy: 13th, 18th, and 21st.

The test was validated for women with unique and twin pregnancies, with at least 10 weeks of pregnancy. This test evaluates just the chromosomal aneuploidies of the 13th, 18th, 21st, X, and Y. In the case of pregnancy with more fetuses, informations about the sexual chromosomes will not be provided. This procedure does not replace the invasive prenatal diagnosis (choriocentesis or amniocentesis).

Also, the analysis cannot highlight the equilibrated chromosomal rearrangements, partial abnormalities of the analyzed chromosomes, structural chromosome abnormalities, fetal or placental chromosomal mosaicism in a small percentage (the presence of two cellular lines with a different set of chromosomes, with a cellular line weakly represented), point mutations, methylation defects, and polyploidy. In particular, the analysis does not reveal the presence of hereditary genetic disorders with the Mendelian transmission. The test can give a false-positive result in the case of chromosomal mosaicism, but this thing can limit itself to the placenta. The sex of the fetus is mentioned as masculine or feminine, based on the presence or absence of the Y chromosome, not on the presence or absence of the SRY gene.

Pregnancies with abnormal ultrasound results should be studied with other types of prenatal investigations, such as fetal karyotype from the chorionic villi or amniotic liquid. Although this test is very precise, the results are not a diagnosis, and they should be evaluated in the clinical context of the patient and the family medical history. The test is limited to offer just informations about the risks of aneuploidies for the analyzed chromosomes.

A "low-risk" result can significantly reduce the possibility for the fetus to present an aneuploidy of the examined chromosomes, but it cannot guarantee that these chromosomes are normal or that the fetus is healthy.

This test can be done for 13th, 18th, and 21st aneuploidies also in case of twin pregnancies and of pregnancies obtained by fertilization in vitro.

This study seeks the degree of accuracy of the prenatal screening which indicates a high risk. The results are established after the confirmation or information of the diagnosis by the invasive diagnosis method for genetical abnormalities, the amniocentesis procedure. The interventions have been made by the obstetrician, helped by different doctors, based on the "2 operatives" technique. The puncture and the extraction of the amniotic liquid will be done by the obstetrician, and the doctor helping him would monitor by ultrasound the whole intervention.

Our study is based on a 3-year research (2012–2015). We evaluated 6097 pregnant women, from which 408 had an indication for effectuation of amniocentesis with the purpose of establishing a correct diagnosis. The dynamics of the number of pregnancies in the analyzed period presents significant decreases from 15%–14.65% in 2014 to 11.15% in 2015.

From the total amount of 408, 114 were made in the year 2012, 102 in the year 2013, 97 in the year 2014, and 95 in the year 2015.

Year	Number of pregnancies	Number of amniocentesis
2012	1641	114
2013	1596	102
2014	1402	97
2015	1458	95
TOTAL	**6097**	**408**

Comparing the dynamics of the number of amniocentesis procedures done in 2015 with the amniocentesis procedures done in 2012, they have a pronounced decreasing rhythm, so that in 2013 the level decreased by 10.53%, in 2014 the level decreased by 14.91%, and in 2015 the level decreased by 16.67% due do the apparition of the Panorama Test sampled from the maternal blood.

Year	Number of pregnancies	Number of amniocentesis procedures	Dynamics than 2012	
			Number of pregnancies	Number of amniocentesis procedures
2012	1641	114	–	–
2013	1596	102	97.26	89.47
2014	1402	97	85.44	85.09
2015	1458	95	88.85	83.33
Total	**6097**	**408**	–	–

By the fact that, in medical practice, age above 35 years old in pregnant women represents a risk factor; the patients have been divided in two groups such as patients aged until 35 years old and patients above 35 years old.

Group of age	2012	2013	2014	2015	TOTAL
Under 35 years old	74	66	57	54	251
Above 35 years old	40	36	40	41	157
Total number of patients	**114**	**102**	**97**	**95**	**408**

In the year of our study, 408 amniocentesis procedures have been made. Two hundred and fifty-one procedures were done on patients with age under 35 years old, which represents a percentage of 61.5%. One hundred and fifty-seven procedures were made on patients with age above 35 years old, with a percentage of 38.5%.

The distribution of the patients based on the pregnancy period and the number of amniocentesis procedures done in percentage:

Weeks of pregnancy	2012	2013	2014	2015	Total	%
Week 15	15	15	14	14	58	14.22
Week 16	36	34	32	39	141	34.56
Week 17	31	28	26	24	109	26.72
Week 18	14	13	IO	8	45	11.03
Week 19	9	5	10	6	30	7.35
Week 20	7	5	3	2	17	4.17
Week 21	2	2	2	2	8	1.96
Total	**114**	**102**	**97**	**95**	**408**	**100%**

Even though the number of amniocentesis procedures has slightly decreased, the number of BI-TESTS has significantly increased since year 2012. In year 2013, the increasing number has been a percentage of +115.4%, in 2013 it has been +315.45%, and in the year 2015, it has been 269.2%. In the last 2 years, the percentage for positive tests for Down syndrome in amniocentesis has been 50%. In all our period of research, 35.05% of the patients have effectuated the double-positive test for the Down syndrome.

Year	Number of amniocentesis procedures	Triple Test positive for Down syndrome	Triple Test positive for Edwards syndrome	Percentage of positive Triple Test (minus the number of amniocentesis procedures) for:	
				Down syndrome	Edwards syndrome
2012	114	93	5	81	4.39
2013	102	64	3	62.75	2.94
2014	97	36	1	37.11	1.03
2015	95	33	1	34.74	1.05
Total	**408**	**226**	**10**	**55.39**	**2.45**

In the period of our research, the percentage for Down syndrome and Edwards syndrome of the TRIPLE TEST decreased in relation to the number of amniocentesis procedures. In this period the result positive for these tests is considered:

- In 2012 the test for Down syndrome represented 81.58%, and 4.39% for Edwards syndrome.

- In 2013 the test for Down syndrome represented 62.75% and 2.94% for Edwards syndrome.

• In 2014 the test for Down syndrome represented 37.11% and 1.03% for the Edwards syndrome.

Although in 2012 the highest number of amniocentesis procedures (114) was recorded, a single case was recorded in which amniocentesis had as an indication the maternal age, which represents a percentage of 0.88%. In 2013, a slight increase with a percentage of 6.86% is noted (in 2014, 4.12%, and in 2015, 3.16%). In all, there were 15 procedures made after the indication of the maternal age above 35 years old, with a percentage of 3.68%.

Year	Number of amniocentesis procedures	Age of the mother >35	Percentage %
2012	114	1	0.88
2013	102	7	6.86
2014	97	4	4.12
2015	95	3	3.16
Total	**408**	**15**	**3.68**

The distribution of the amniocentesis procedures based on priors:

• Down syndrome in the family

• Prior pregnancy with Down syndrome

• Prior pregnancy with Turner syndrome

Year	Number of amniocentesis procedures	Down syndrome in the family	Prior pregnancy with Down syndrome	Prior pregnancy with Turner syndrome
2012	114	1	1	0
2013	102	0	0	0
2014	97	1	0	1
2015	**95**	**0**	**0**	**0**

It is observed that in 2012, from the amount of 114 amniocentesis procedures, 2 of them had as indication the pathological chromosomal history. One of the patients presents in her family history a member of the family who has Down syndrome. Another patient had a prior pregnancy with Down syndrome. These indications represent 1.75% of the indications for year 2012. In 2014 two more pregnant women have been diagnosed. One of them was coming from a family with Down syndrome, and the other one had a prior pregnancy with Turner syndrome. These indications represent a percentage of 2.61% for the year 2014.

From a total of 408 pregnancies, the indications for pathological chromosomal history represent a percentage of 0.97%.

Number of amniocentesis procedures	408	100%
BI-TEST	131	32.10%
TRIPLE TEST	258	63.23%
Indication for maternal age 35 years or above	15	3.67%

Down syndrome in the family history	2	0.49%
Prior pregnancy with Down syndrome	1	0.24%
Prior pregnancy with Turner syndrome	1	0.24%

From the amount of 408 pregnancies which underwent the amniocentesis procedure, 149 of them were on their first pregnancy, with a percentage of 36.52%. One hundred and ninety-eight of the pregnant women were on their second pregnancy (48.53%), and 61 were on their third or more pregnancy (14.95%).

Distribution of the patients depending on environmental residence

Environment	2012	2013	2014	2015	Total	%
Rural	16	34	25	20	95	23.28
Urban	98	68	72	75	313	76.72
Total	**114**	**102**	**97**	**95**	**408**	**100**

It is concluded that only 23.28% of the pregnant women came from a rural environment; the rest of 76.72% came from an urban environment.

Distribution of patients depending on chronic diseases

Chronic diseases	Number of patients	%
Diabetes mellitus	11	2.70
Hepatitis A and hepatitis B	5	1.23
High blood pressure	9	2.21
Obesity	10	2.45
Kidney diseases	3	0.74

Distribution of patients depending on vicious behavior

	2012	2013	2014	2015	Total	%
Smoker	38	49	36	37	**160**	**39.22**
Non-smoker	76	53	61	58	**248**	**60.78**
Total	**114**	**102**	**97**	**95**	**408**	**100**

It can be observed that, despite the fact that smoking affects the pregnancy and the fetus, a quite high percentage of pregnant women smoke (39.22%).

Distribution of the patients depending on the result of the amniocentesis

Result	Number of patients	%
Normal results	394	96.57
Down syndrome	10	2.45
Edwards syndrome	4	0.98
Total	**408**	**100**

The percentage of Down syndrome reported to the total number of pregnancies (6097 patients) represents 1.6% above the European statistics. The percentage of Edwards syndrome reported to the total number of pregnancies represents 0.656% above the European statistics.

Maternal ages for women diagnosed with pregnancies with Down syndrome were 28, 31, 32, 38, and 40 years old.

Age of the mother diagnosed with pregnancies with Down syndrome	Number of cases
28	2
31	1
32	2
38	3
40	2

All the patients with babies diagnosed with Down syndrome or Edwards syndrome decided to terminate the pregnancy.

The 14 cases positive for Down syndrome and Edwards syndrome (4 cases positive for Edwards syndrome and 10 cases positive for Down syndrome) were actually found because of the indications of the TRIPLE TEST, 4 more cases positive for Down syndrome were found because of the indications for BI-TEST, and just 1 case of Down syndrome was found because of the indications for maternal age.

Amniocentesis has become the most used prenatal diagnosis method for women aged 35 and above or even for younger women with a family history specific for hereditary congenital abnormalities.

In our studied group, 157 pregnant women asked for a prenatal diagnosis for Down syndrome. They were aged 35 or above 35 and demanded the association of some pathological biochemical markers (double test, TRIPLE TEST).

The frequency of the structural chromosomal abnormalities (translocations, deletions, inversions) did not show a similar numeric relationship regarding the maternal age.

According to the literature, the screening tests have a margin of error in the diagnosis of fetal malformations, so the diagnosis of certainty will result after the amniocentesis.

The majority of the specialists consider that amniocentesis is an invasive procedure, so it is recommended that the number of these procedures should be decreased. In this regard, our study did very well: the result of the utilization of the other methods (BI-TEST, TRIPLE TEST) was positive. The BI-TEST can detect trisomy 21 (the Down syndrome) and trisomy 18 (the Edwards syndrome).

There are tests like Panorama Test which are cutting-edge technology, and they reduce the use of amniocentesis. But the cost of this test is high and not covered by the National Health, which is the reason why it is not accessible for all patients.

Our study has been made on a large segment of the population which had access to all the screening tests (BI-TEST, TRIPLE TEST), but not all of the patients had this possibility.

The TRIPLE TEST in pregnancy can help in tracking down the neural tube defects and chromosomal abnormalities such as trisomy 18 and trisomy 21. So, if a high level of alpha-fetoprotein (AFP gene) is found in the blood, it is likely that the fetus presents a neural tube defect (more exactly the brain and the spinal cord), defects such as spina bifida or anencephaly. If the results of the TRIPLE TEST show a low level of alpha-fetoprotein (AFP gene) and estriol, it can indicate trisomy 21 (the Down syndrome), trisomy 18 (Edwards syndrome), or other chromosomal abnormalities.

Before we proceeded with the amniocentesis procedure, we acquired from every patient and her partner a written consent after informing them about what the procedure implies.

In obstetrical practice the risk of carrying out an amniocentesis is evaluated permanently, compared to the individual risk of numerical chromosomal abnormalities or structural chromosomal abnormalities. We recommend the amniocentesis as an invasive diagnosis prenatal method when the risk of a detectable abnormality is higher than the risk of the procedure itself.

It is important that the patient should be informed about the pam felt during the amniocentesis.

Numerical chromosomal abnormalities (aneuploidies)—in the case of amniocentesis procedures done to pregnant women with age 35 or above from our consignment—have a frequency of 3.18% (5 cases out of 157).

The majority of the patients questioned said that the procedure was a painful method. Pain is a multidimensional sensation and complex, which varies in quality, intensity, duration, localization, and inconvenience from an individual to another. The intensity and inconvenience of the pain is not directly connected to the nature and the extension of the tissue lesion. Cultural, genetic, familial, growth, psychological, and social variables have a strong impact in the perception of the pain and in its expression from the patient.

The objective quantification of the pain is difficult. Before any invasive procedure effectuated throughout pregnancy, the biggest concern of the patient is the risk and the intensity of the pain during procedures.

Ultrasound conclusions in cases of chromosomal abnormalities

Trisomy	Ultrasound conclusions
Trisomy 21 (Down syndrome)	• Duodenal atresia, tracheoesophageal fistula, and hydramnios are present usually if there are gastrointestinal damages • Heart abnormalities—defects of the atrial septum • Hypoplasia of the middle phalanx of the 5th finger of the hand • Conclusions from the second trimester: increased cervical skinfold (>6 mm), ratio of the actual femur (length), and the expected femur = 0.91
Trisomy 18 (Edwards syndrome)	• Growth retardation in uterus • Hydramnios • Hand wrapped with superimposed fingers (the index covers the third finger, and the fifth finger covers the fourth finger) • *Ankle equinus* with prominent calcaneus and clubfoot. • Heart abnormalities: ventricular septal defect • Omphalocele, diaphragmatic hernia • Cyst of the choroid plexus
Trisomy 13 (Patau syndrome)	• Holoprosencephaly (defects of the facial break line) • Cleft palate • Heart abnormalities such as ventricular septal defect • Polydactylism • Omphalocele • Polycystic kidneys

6. Conclusions

In conclusion, all the pregnant women who underwent the amniocentesis procedure and had a negative result had an on-term pregnancy and delivered healthy newborn babies.

As for the pregnant women who underwent the amniocentesis procedure and had a positive result, all of them decided to terminate their pregnancy due to existent fetal malformations.

The purpose of our study was to decrease the use of amniocentesis procedures and to introduce a cutting-edge technology, which is the Panorama Test for early diagnosis of fetal malformations. We tried to implement a noninvasive method (Panorama Test made from the mother's blood sample) over an invasive method which is the amniocentesis.

Our purpose has been achieved by the decrease of amniocentesis and by increasing the use of cutting-edge technology without affecting the final results in the percentage of the fetal malformations statistics worldwide.

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Section 3

Cancer Genetics

Genetic Contributors to Hereditary Cancer Predispositions: Do We Have Enough Information?

Tom Nolis and Rodney J. Scott

Abstract

Genomics medicine and molecular genetics are experiencing a surge of interest as well as a push for a more prominent role in mainstream medicine. This, coupled with the advancement of next-generation sequencing, along with a national, if not global, steering of funding to support the advancement and development of genetics is suggesting that we are entering a new age of medicine. As this push begins to gain some momentum, the impact of genomics medicine on clinical utility and the influence of supporting data on genes that make their way from research to diagnostic medicine are worth reviewing.

Keywords: genetics, genomics, utility, cancer, *BRCA1*, *BRCA2*, *PALB2*, population, family, epidemiology, multigene, panels

1. Introduction

One of the most fundamental clinical validity and clinical utility questions currently at the forefront of molecular genetic testing is as important today as it was nearly 30 years ago, "is the genetic variation detectable in the genes of interest actually associated with a clear and quantifiable risk for disease?" or in other words, "are the variants that we are finding in these genes relevant for the disease of interest?"

The question above is a very important one, and to be able to answer, it we must take a step back and explore clinical utility and epidemiology in genetics more thoroughly. The answer to this question requires an inevitable focus on cancer genetics as breast and ovarian cancer are excellent examples of both past and present accomplishments in genomics medicine. These disease entities are topical and have enough data to appropriately highlight the genetic journey previously taken into genomics medicine, and they are also able to shed light on how new genetic players are entering the diagnostic scene (e.g., *PALB2*).

Finally, the following text will briefly compare and contrast the significant influence of family-based and population studies on genetic data. This chapter will close on a more general note by reviewing the current cancer assessment guidelines and how these reflect the current clinical utility of genomics medicine.

1.1 Key points

- Clinical utility in genetics is largely a continual revamp of the ACCE framework.

- Genetic epidemiology has followed a natural flow paralleling the advancement of technology and detection.

- Family-based studies and population data are still at the forefront of both research genetics and diagnostic genomics.

- *BRCA1* and *BRCA2* are excellent models for useful genetics input.

   ○ This has led to a complete change in the identification and treatment of a very tangible cancer entity.

- Less penetrant genes in breast and ovarian cancer, such as *PALB2*, along with multigene panels have their place in genomics medicine, but, generally, quality supportive data are lacking.

2. Clinical utility in genetic testing

Clinical utility is a broad concept and one that is deeply fundamental in the world of medicine. At its most basic level, it is the essence of what propels medical advancement. It answers the question of "what should I do next?" when this question is asked by physicians, but it can also be a much larger almost all encompassing concept; as if to ask, "what is worth doing next?" in any field of medicine. Clinical utility, by many sources, can be simply defined as "the balance of benefits to risks" or more broadly refer to any use of test results to inform clinical decision-making [1]. A genetic test can be defined as a "test that involves the analysis of chromosomes, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), genes, or gene products (e.g., enzymes and other proteins) to detect heritable or somatic variations related to disease or health. Whether a laboratory method is considered a genetic test also depends on the intended use, claim or purpose of a test" [2].

In some fields, such as in colorectal surgery, it is easy to determine clinical utility: if the patient's morbidity or mortality is improved by removing a tumor, then you remove it; otherwise you do not. In the realm of genetic testing, the concept of clinical utility can be difficult to precisely define—or even quantify. Indeed, this was reinforced when experts in evidence-based medicine and genetics for the Centers for Disease Control and Prevention (CDC) in 2005 failed to come to a consensus on the term "clinical utility" despite initially claiming to be confident in its meaning beforehand [1]. As a consequence, the term had to be elaborated on by the Analytic validity, Clinical validity, Clinical utility, and Ethical, legal and social implications (ACCE) project that was carried out by the Foundation for Blood Research with support from the CDC [1]. In screening or in diagnostic testing, clinical utility broadly refers to the ability of a test to prevent or ameliorate adverse health outcomes such as mortality, morbidity, or disability through the adoption of efficacious treatments conditioned on test results (**Figure 1**) [1]. The perceived value of genotypic information, at this point in time, includes a more thorough understanding of a patient's diagnosis, prognosis, risk, and disease or treatment susceptibility; the caveat is that this knowledge may not influence clinical management at all. Clinical utility in the ACCE framework was expanded to include
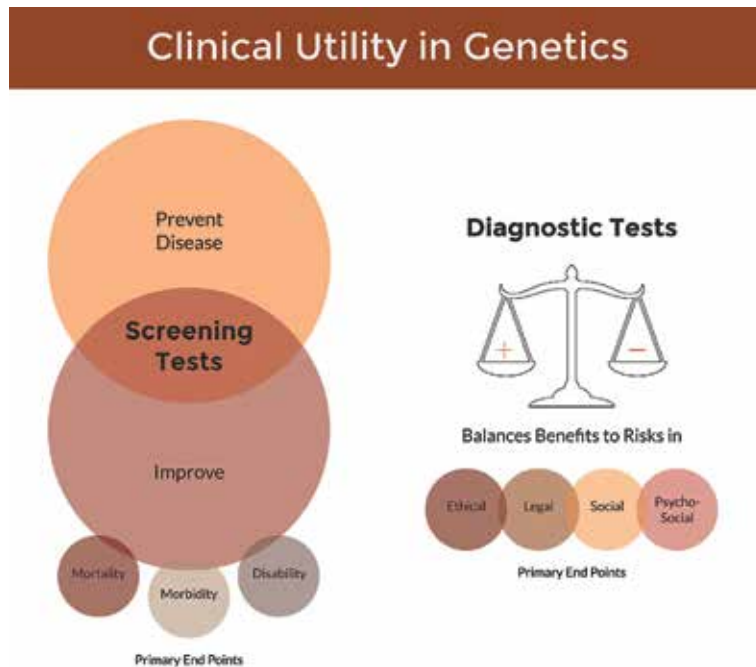
Figure 1.
Clinical utility in genetics. The clinical utility in screening tests is to provide preventative care and to improve primary end points such as overall morbidity; in a diagnostic setting, the value of genetic testing lies in the balance of benefits to risks in more psychological domains.

contextual or implementation issues (e.g., availability of resources) and that clinical utility can also include psychological benefits.

2.1 Evaluating genetic tests

Evaluating genetic tests is often methodologically difficult largely due to small patient populations and the resulting dearth of high-quality studies. Adapted from Morrison et al. 2012, the following subsections are generally agreed upon as common characteristics for reviewing genetic tests [3, 4].

2.1.1 Overview of disease and underlying genetics

- Information on the disease prevalence, treatments, and outcomes of the disease as well as overall cost

- Description of the genetic causes, including inheritance patterns

- Classifying the mutational spectrum, along with the prevalence and penetrance

- Determination if there is any "gold standard" tests to compare to

2.1.2 Target population and intended use

- Prevalence for target population involves age, ethnicity, and eligibility for testing

- Purpose of the test: is it diagnostic, for treatment, prognosis, management, carrier testing, prenatal testing, or other?

2.1.3 Laboratory information

- Validation details

  - Test new or already in use?

    - If already in use:

      - Number and rate of positive and negative mutations

      - Turn-around-time for results

      - Similar tests available

      - Current testing activity and expected with appropriate funding figures

      - Whether there are other laboratories that could offer the test

      - Infrastructure requirements

- Quality assurance, maintenance, and improvement programs—both internally and externally

2.1.4 Economic considerations

- Cost estimates for the test including equipment, personnel, and consumables/ reagents

- Costs of disease burden with or without treatment

- Costs saved by employing test

2.1.5 Analytic validity

- Precision, reliability, accuracy, sensitivity, and specificity of the genetic test, and how these compare with other employed screening and diagnostic tests

2.1.6 Clinical validity

- Specificity and sensitivity, positive predictive value, negative predictive value, likelihood ratios, and how these compare with other employed screening and diagnostic tests

2.1.7 Clinical utility

- Benefits and risks of the test

- Treatment of the patient

- Patient's health outcome

- Effect on patient and patient's family members

- Alternative diagnostic tests compare and contrast to genetic testing

2.1.8 Ethical, legal, and social

- Details on ethical, legal, and societal issues include support and follow-up

2.2 ACCE model and beyond

The CDC furthered the ACCE framework by establishing the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Initiative. The EGAPP supports the development and implementation of an evidence-based process for evaluating genetic tests and other genomic applications for clinical and public health practice. An independent, nonfederal EGAPP Working Group (EWG), consisting of a multidisciplinary expert panel selects topics of interest, reviews evidence, and recommends courses of action [2]. Key objectives of the EWG are to develop an openly accountable process, reduce conflicts of interest, and provide a connection between well-established evidence and the EWG recommendations [2, 5].

The ACCE model has stood the test of time and has proven to be a basis for the technical appraisal of genetic tests across the globe (**Figure 2**). The UK Genetic Testing Network and the Andalusian Agency for Health Technology Assessment expanded the ACCE model to guide the introduction of new genetic tests into their
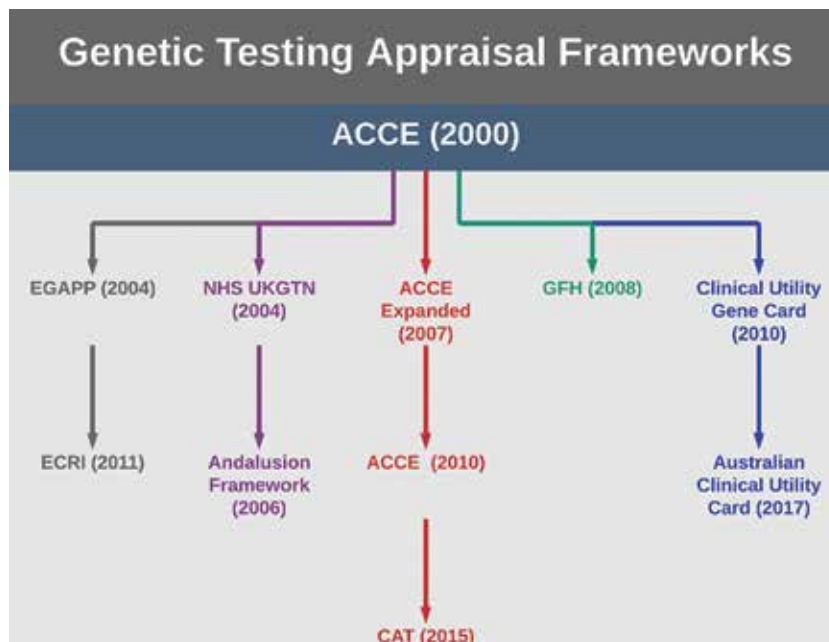

Figure 2.
Genetic testing appraisal frameworks. Testing appraisal frameworks around the globe have largely stemmed from the analytic validity, clinical validity, clinical utility, and ethical, legal and social implications (ACCE) framework announced in 2000. EGAPP, evaluation of genomic applications in practice and prevention; ECRI, Emergency Care Research Institute; NHS UKGTN, National Health Service UK Genetic Testing Network; CAT, companion test assessment; GFH, German Society of Human Genetics.

public health system, creating the 2004 NHS UKGTN Gene Dossier and the 2006 Andalusian Framework. In 2007, the ACCE model was again modified by adding health quality measures to the evaluation process. The process was made more streamlined, shortening the systematic review process for emerging genetic tests. In 2010, the ACCE model was adapted to particular types of genetic testing through the Complex Disease Framework and the ACHDNC Newborn Screening Framework of the Advisory Committee on Heritable Disorders in Newborns and Children. The ACCE model also spawned two European frameworks: the 2008 GFH Indication Criteria of the German Society of Human Genetics and the 2010 Clinical Utility Gene Card of EuroGentest. The 2015 Companion test Assessment Tool (CAT) build upon the ACCE model to determine which tests needed further evaluation. In 2011, the ECRI Institute utilized the EGAPP process to develop a set of analytical frameworks for various testing scenarios and other stakeholder aspects. Lastly, the EuroGentest inspired the 2017 Australian Clinical Utility Card [5].

We cannot ignore the influence of the stakeholders who will invariably have wildly different opinions as to which outcomes are considered relevant. Thus, the types of outcomes that must be considered in evaluating the utility of a genetic test will also depend on the purpose of the test and the congregation of those who make the final decision. For example, a state-funded public health program will likely focus on the overall impact on morbidity and mortality versus privately funded sectors concentrating on the greater net profit. Coverage decisions by third-party payers may be based in large part on perceptions that test results are useful for timely or accurate diagnoses and clinical management. For a test that is offered to families in a clinical setting on a voluntary basis, the value of information for making career, residential, and reproductive decisions take on greater relevance.

Before a genetic test can be accepted into clinical practice, data must be collected to demonstrate the benefits and risks that accumulate from both positive and negative results [1]. Referring back to the colorectal surgery example above, the term "clinical utility" fits in very well; however, when applied to genetics or genomics medicine, the term may be too limited. The clinical end points are extremely important, but the utility or actionable context must remain broad in genetics to embody an overall net benefit. Genetic testing is particularly useful in the psychosocial, ethical, legal, and social (ELS) realms as well as in many diagnostic cases (**Figure 1**). By including clinical and ELS together in the concept, improvements in health outcomes such as enhancements in morbidity, mortality, and in disability become strong primary end points when assessing the utility of genetic testing.

3. Genetic epidemiology: population and family-based studies

The process of genetic epidemiology has flowed sequentially from observing phenotypic differences between populations to demonstrating that diseases can run in families, to examining feasible genetic susceptibility models, to tracking co-segregating genetic markers with disease in families, to narrowing the region of candidate genes, to association analyses with candidate genes, to cloning and mutation identification, to functional and structural characterization of a gene, and, ultimately, to extending the phenotype characterization even further based on the genotype identified (**Figure 3**) [6].

Population and family-based studies are at the heart of genetic epidemiology. Population-based association studies are generally regarded as more statistically powerful than family-based studies, as they tend to have more subjects and are overall easier to execute [6]. Family-based designs are influential methods that use the proband as well as their relatives to assess the genetic and molecular

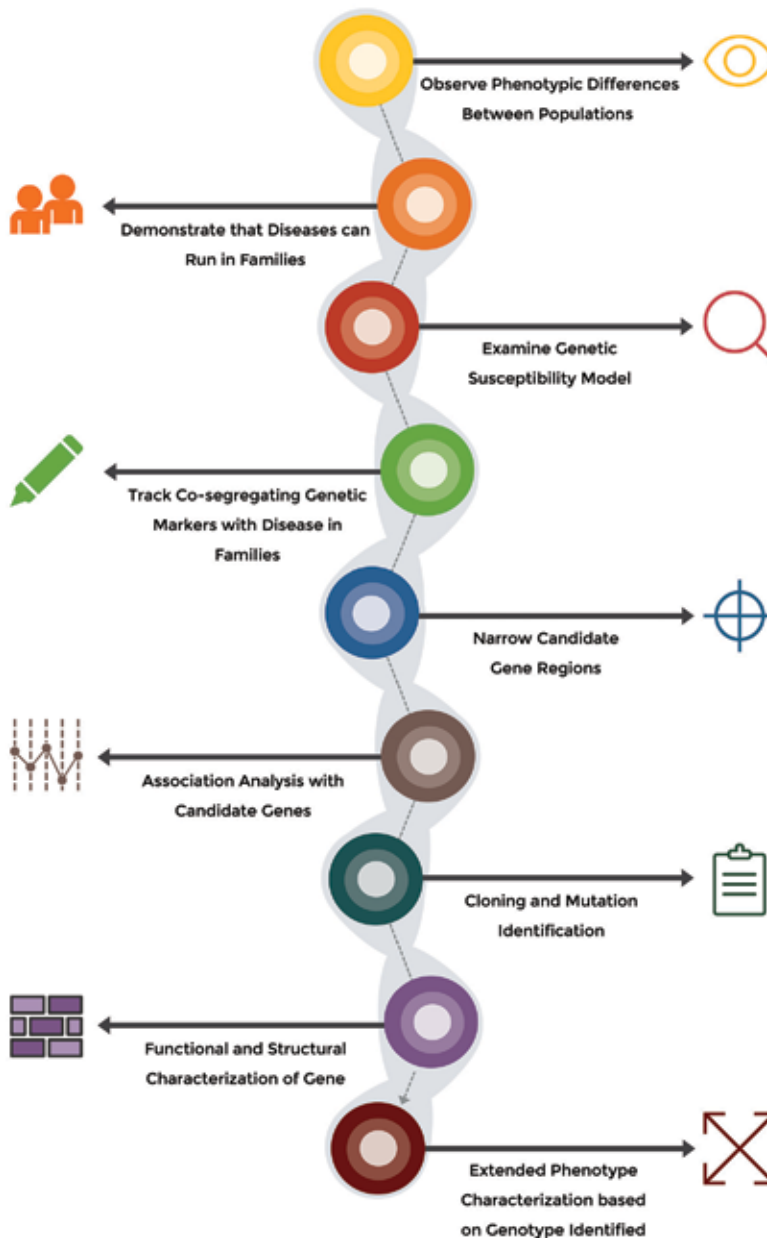# Genetic Epidemiological Historical Progression

Figure 3.
Genetic epidemiological historical progression.

epidemiology of disease (**Figure 4**). The various types of studies available include familial aggregation, twins, segregation, linkage, and association.

Linkage and association studies directly evaluate genetic markers and require the collection of DNA from the study subjects—as opposed to twin studies, segregation studies, and familial aggregations studies. Family-based studies have been the long-standing primary approach to detect disease-causing genes. Segregation and linkage studies are highly valuable methods for assisting in
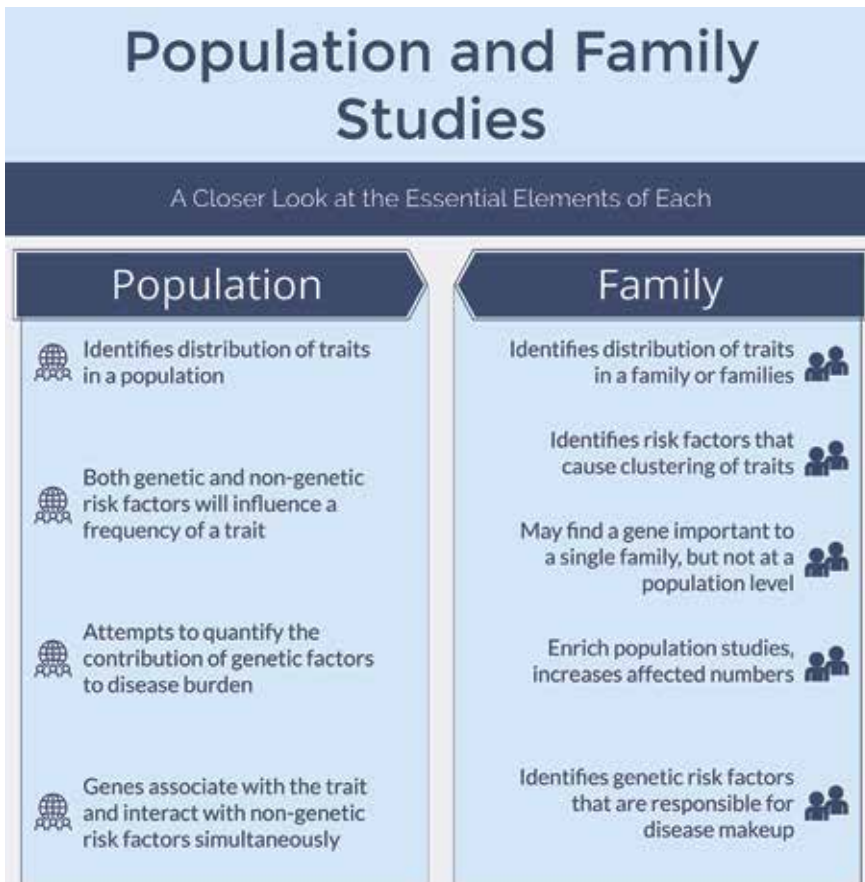
Figure 4.
A closer look at family-based and population studies.

cloning highly penetrant rare disease-causing genes. Family-based association studies strengths lie in their ability to control for confounding bias due to population stratification—albeit they do so at a loss of power [1, 5, 6].

Population stratification is when contrived associations can be detected if cases and controls come from different source populations that have systematic differences in ancestral allele frequencies. A great advantage of employing family-based study designs is that population stratification can be circumvented. By studying parents and their offspring/siblings, cases and controls within each family are virtually guaranteed to arise from the same sample source or population. Due to the increasing efficacy of identification of association with disease, the importance of family-based studies has seemingly subsided; however, it is worth pressing that family-based studies are arguably more important than they have ever been as they are still the only way to truly link a causative variant to disease [1, 5, 6].

Family-based studies can help determine whether a disease or trait is genetically influential by studying familial aggregation. Results can be furthered with a segregation analysis to identify the mode of inheritance. The results from the segregation analysis can add power to a linkage analysis, which searches across the entire genome in an attempt to locate regions containing causal genes [1, 5, 6].

Segregation analysis is a method of establishing the genetic inheritance of disease and is performed exclusively with family data. This approach assists in

determining if a disease segregates with a variant in a single gene; in addition to this, the mode of inheritance can also be ascertained. Very large pedigrees and families with a plethora of affected individuals are exceptionally informative for identifying specific genes [1, 5, 6].

Once a family is collected, and studied, in a segregation analysis, that family is generally made available for further analyses (e.g., linkage). Linkage occurs when two loci/alleles on the same chromosome are inherited together. Since recombination during meiosis can occur virtually anywhere in the genome it stands to reason that the closer two loci are to each other the less likely they will be separated after a recombining event (i.e., the more likely they are to stay together after recombination), that is, it says they are "linked." Linkage analyses utilize this phenomenon by investigating co-segregating genetic markers along with a disease trait seen within the family (or families)—the trait can be either qualitative or quantitative. If the markers and traits are observed to co-segregate within families, it can be logically inferred that the disease-causing variants are within close proximity to the markers [1, 5, 6].

This point reveals that linkage is "intrafamilial," whereby the co-segregating marker allele may very well be different in different families. Families are generally recruited into linkage studies on the basis of having at least one identified affected individual, and the families are either quite large or have affected siblings. Generally, the markers are spaced evenly over the entire genome, and linkage can be performed by utilizing these markers.

4. Breast and ovarian cancer: the journey of finding *BRCA1* and *BRCA2*

Establishing penetrance is an arduous undertaking even for some of the most well-studied genes (e.g., with *BRCA1* and *BRCA2*) simply because no two genetic studies have yielded the same findings. Many of the data and results discrepancies can be linked to differences in the populations studied and to the methodologies employed. It is vanishingly rare, if not impossible, for these studies to have similar methodologies to allow for perfect reproduction of results. The range of penetrance found for the BRCA variants and genes has guided the clinical recommendations for breast and ovarian cancer surveillance and prevention and has provided a sort of genetic "gold standard" by which all other genes are now compared and contrasted against [7, 8].

A strong family history of breast cancer is associated with an early age of onset, the addition of ovarian cancer, bilateral tumors, and a rarely affected male. *BRCA1* (and similarly for *BRCA2*) has been identified through the study of women who have presented with a strong family history of breast and ovarian cancer (**Figure 5**). In 1988, more than 1500 families with multiple cases of breast and ovarian cancer were studied; the data generated was subjected to a segregation analysis. The results illuminated that roughly 5% of cases, particularly those with early-onset disease, could be heritable in a Mendelian fashion. Selected families, those with near-Mendelian pedigree patterns, were aggregated for linkage analysis. A major susceptibility locus, *BRCA1* (OMIM 113705), was mapped to 17q21 in 1990. 17q-linked families that were above 45 years of age at diagnosis were given negative LOD scores.

In 1994, a linkage analysis performed on 15 large families with breast cancer that were also determined to not be linked to 17q helped identify the *BRCA2* locus located on 13q12 (OMIM 600185). Later that year *BRCA1* was cloned, and in 1995 *BRCA2* was cloned. *BRCA1* variants accounted for a very large proportion of
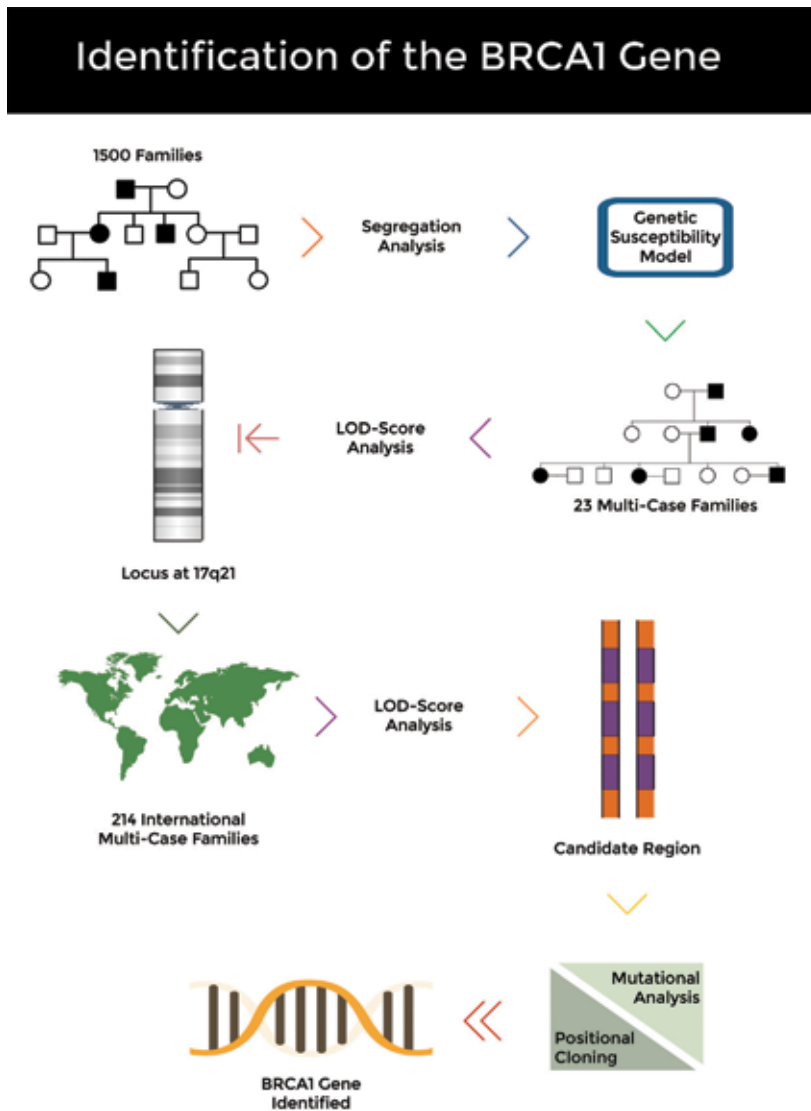
Figure 5.
The progression of discovering the BRCA1 gene.

families with both breast and ovarian cancers, while male breast cancer was predominately observed in *BRCA2* variant families [9].

  BRCA1 and *BRCA2* were identified by focusing on a small number of specific families. Other candidate genes were established from focused studies.

- In 2004, the variant c.1100de1C in the *CHEK2* gene was observed in 201 cases (1.9%) and 64 controls (0.7%) in 10,860 breast cancer cases and 9065 controls from 10 case-control studies in 5 countries (estimated odds ratio (OR) 2.34; 95% CI 1.72–3.20; P = 0.0000001) [10].

- In 2006, the *ATM* gene was screened in individuals from specific families, and 12 mutations were found in affected individuals and in 2 controls (P = 0.0047) from 443 familial breast cancer pedigrees and 521 controls (estimated relative risk of 2.37 (95% CI 1.51–3.78, P = 0.0003) [11].

- *BRIP1* was initially described as a breast cancer predisposition gene in 2006. The analysis of 1212 women with familial breast cancer along with 2081 controls yielded mutations in 9 cases and in 2 controls (estimated relative risk of 2.0; 95% CI = 1.2–3.2; P = 0.012) [12].

- *PALB2* mutations were found in 10 cases of 923 individuals with familial breast cancer, and no mutations were found in 1084 controls (P = 0.0004) (estimated odd ratio of 2.3; 95% CI = 1.4–3.9; P = 0.0025) [13].

These factors are the most relevant in families where the disease and the variant are actually segregating together, but at a population level, their overall implication is surprisingly small. For instance, *CHEK2*:c.1100de1C, at a population level, is seen in only 1.9% of cases [14].

5. Less penetrant genes: *PALB2*, *BRIP1*, and *RECQL*

Traditionally, epidemiological studies tend to emphasize the inclination that the greatest benefits to the population are found in interventions that decrease risk factors for the bulk of the population, not in targeting a small number of individuals at the extreme ends of the risk spectrum. As we can see from the sample multigene panel for hereditary breast cancer the genes selected for testing range from quite influential (e.g., *BRCA1* and *BRCA2*) to marginally influential (e.g., *STK11*) (**Table 1**). However, it is quite interesting to explore the data for lower-risk genes such as *PALB2* and *BRIP1* and also for genes that are suspected of having involvement in breast or ovarian cancer, but the data is not quite yet congruent with the theory (e.g., *RECQL*).

Less penetrant genetic loci are mainly represented by the single-nucleotide polymorphisms (SNPs) or by the variants of uncertain significance discovered through genome-wide association studies. Variants associated with a minor increased risk, unlike high-risk mutations, can only account for a small portion of cancers seen in family histories of carriers. Thus, the cumulative risk for carriers with a positive family history will largely depend on the risk levels attributed to both their family history for that cancer and the risk induced by the variant itself. For example, if a carrier of a variant associated with an increased odds ratio of cancer also has a family history sufficient to quadruple her risk, her cumulative risk will be about that of a woman with a 4 x OR increased risk [7].

Mutations in *ATM*, *PALB2*, and *CHEK2*:c.1100delC, in conjunction with a strong family history, are very likely to be associated with a high absolute risk of breast cancer [17]. It is important to note that the family history creates a context whereby it changes the penetrance of these mutations. This interpretation is clinically important and would justify testing for these mutations in multi-case breast cancer families such as those seen by typical cancer family genetics services.

Genetic risk factors, which are familial by their very definition, will be more frequent in women with positive family histories involving multiple breast cancers through their direct association with breast cancer as a disease entity and with their direct association with the familial aspect of breast cancer. This can be illustrated in a simple example where we consider a rare mutation whose presence doubles the risk of breast cancer relative to the general population: it will be roughly four times more common in women who are affected and who have an affected first-degree relative, which will square the ratio [17].

Sample multigene hereditary breast cancer panel	
Gene	**Notes**
ATM c.7271 T > G	—
other *ATM* mutations	Lack of data regarding penetrance and surveillance except for c.7171 T > G mutation. If mutation has been identified, a careful assessment of residual risk for relatives who are noncarriers is needed
BARD1	—
BRCA1	—
BRCA2	—
BRIP1	—
CDH1	Increased risk of lobular breast cancer. Gastric cancer risk is unknown when mutations are identified in absence of a positive family history of gastric cancer
CHEK2 c.1100delC	Mutations are rare, but high penetrance in some families
other *CHEK2* mutations	Lack of penetrance data except for in specific mutations
NBN	—
NF1	Can identify mutation carriers by clinical phenotype and then perform gene specific test
PALB2	Mutations are rare, but high penetrance in some families
other *PALB2* mutations	—
PTEN	Can usually be identified by clinical phenotype
RAD50	—
STK11	Can usually be identified by clinical phenotype. Low penetrance in breast cancer
TP53	Breast cancer risk management inferred from other genes. High penetrance for breast cancer, but mutations are rare
Adapted from: Invitae and eviQ [15, 16].	

Table 1.
Sample multigene panel offered for hereditary breast cancer.

5.1 PALB2

The Finnish founder mutation was found in 0.9% (18 of 1918) of cases without selecting for or emphasizing family history. Likewise, a French-Canadian founder mutation was found in 0.6% (2 of 356) of cases also without selecting for or emphasizing family history selected. The numbers of the *PALB2* founder mutation carriers were too small to make precise risk inferences, but modified segregation analyses of data from the families of case-carriers were used to estimate risk for carrier families, which has demonstrated the importance of these founder mutations in the risk of developing breast cancer [13, 14, 18, 19].

Blanco et al. found that the frequency of *PALB2* mutations was ∼1.5% after investigating the incidence of mutations in *PALB2* patients with breast cancer that were also negative for any variants in *BRCA1* and *BRCA2* +/− a family history of pancreatic cancer; previous studies had the mutation rate for similar cohorts range from 0 to 4.8%. Dansonka-Mieszkowska et al. conducted similar research among Polish women; their study showed a minor but significant *PALB2* mutation presence at ∼0.6% [13, 14, 18, 19].

Likewise, Bogdanov et al. conducted a study on the occurrence of *PALB2* mutations among Russian and German women and reported a mutation rate of ∼2%. In 2014, Antoniou et al. tested 362 women from 154 families and found that the risk of breast cancer in women 40 years of age or younger with *PALB2* mutation was ∼9x greater, ∼8x greater in patients aged 40–60 years, and ∼5x greater in patients 60 years and older when compared to the general population. The absolute risk of breast cancer in women with *PALB2* mutations under 70 years of age ranges from 33 to 58% for women without and with a positive family history of breast cancer, respectively. Interestingly, Hartley et al. conducted a study that confirmed that as the number of cases of breast cancer in a family increases, the likelihood of *PALB2* mutations increases as well. Women with 3+ positive cases of familial breast cancer have a 2.6% greater likelihood of having a *PALB2* mutation than those without multiple cases [13, 14, 18, 19].

Southley et al. studied Australian women to determine the occurrence rate and penetrance of *PALB2* mutations. The study found that the women with breast cancer and a positive family history of having *PALB2* mutations had *PALB2* mutations present in 1% of patients. The study identified that the women with breast cancer and no family history of *PALB2* mutations had *PALB2* mutations present in only ∼0.4% of patients. There were no *PALB2* mutations detected in the control/healthy population of women. Heikkinen et al. studied southern Finnish women and found *PALB2* mutations in 2% of patients with a positive familial history of breast cancer and also in 0.6% of women with a sporadic breast cancer presentation [13, 14, 18, 19].

In 2016, a seminal paper by Thompson et al. examined 2000 predominantly breast cancer-affected women with a strong family history that were also *BRCA1* and *BRCA2* variant-negative and compared them to 1997 controls. They observed that a significant proportion of mutations were only in *PALB2* (26 cases vs. 4 controls) and in TP53 (5 cases vs. none in controls), whereas no mutations were identified in *STK11* [14]. *PALB2* is a great example of how penetrance estimates can depend on the population, family history, and age at onset as well as other considerations [17].

5.2 BRIP1

BRIP1 was initially described as a breast cancer predisposition gene in 2006. The analysis of 1212 women with familial breast cancer along with 2081 controls yielded mutations in 9 cases and in 2 controls (estimated relative risk of 2.0; 95% CI = 1.2–3.2; P = 0.012). However, recently *BRIP1*'s association with breast cancer has grown suspect, while its association with ovarian cancer has risen sharply. *BRIP1* mutations confer a high ovarian risk in familial index patients (OR = 20.97, 95% CI = 12.02–36.57; P < 0.0001) and in the subgroup of patients with late onset ovarian cancer (OR = 29.91, 95% CI = 14.99–59.66; P < 0.0001) [12].

5.3 RECQL

In a screen of 144 Polish and 51 French-Canadian women with early-onset familial breast cancer, 2.6% possessed truncating mutations in *RECQL*. Validation studies that reviewed the *RECQL* variant c.1667_1667C3delAGTA in over 13,000 breast cancer patients with 4702 Polish controls showed the *RECQL* mutation appeared in 0.23% of cases and in 0.04% in controls. Likewise, the *RECQL* variant c.634C > T (p. Arg215*) seen in the French-Canadian population was further screened in 538 patients and 7136 newborn controls and was detected in 5 patients and in one control—a nearly 50x increase in frequency in affected versus

unaffected individuals. Studies of patients in northern China revealed a pathogenic *RECQL* mutation in 2.0% of the 448 familial breast cancer patients compared to the 0.06% seen in 1588 control subjects [20]. By whole exome sequencing 0 early-onset familial breast cancer patients without *BRCA1*/2 mutations and by screening the *RECQL* gene in an additional 439 unrelated familial breast cancer patients, 9 index cases were found to carry a pathogenic mutation in the *RECQL* gene among the 448 BRCA-negative familial breast cancer patients. It was determined that the pathogenic mutation rate of the *RECQL* gene in familial breast cancer in *BRCA1*-/*BRCA2*-negative breast cancer patients was 2.0%. Further to these results, no loss of heterozygosity was found in the *RECQL* mutation carriers, suggesting that *RECQL*-associated tumorigenesis is likely through classical haploinsufficiency [21].

In 2018, Li et al. sequenced all the exons of RECQL and at least 10 bp of the exon–intron flanking regions in 9112 subjects from Australia. The case subjects were females diagnosed with breast or ovarian cancer from 4536 families with a negative result after BRCA1 and BRCA2 mutation testing. The controls were 4576 women who were above 40 years of age and were cancer-free as of May 2016. Thirteen loss-of-function mutations in the cases and 25 in the controls were identified (0.29 versus 0.55%, odds ratio 0.52, 95% confidence interval 0.25–1.06, $P = 0.072$ by two-tailed Fisher's exact test) [22]. Missense variants observed between cases and controls were not statistically significant (54 cases, 1.19%, versus 37 controls, 0.81%; $P = 0.073$) [22]. It is generally accepted that a predisposition gene is considered actionable only if the 90% confidence limit of the estimated relative risk is greater than four [22]; therefore, RECQL, can be excluded based on these findings.

5.4 Lack of data and the impact of finding a variant of unknown significance

Despite the intense and widespread shared enthusiasm to reduce the risk of predictable cancers, the adoption of the more recent breast cancer predisposition genes, such as *PALB2*, has been sluggish. This has been largely due to the communal appreciation that following a variant of unknown significance result, the complexity of interpretation may lead to a subsequent clinical utility hindrance. An additional 3.9% of patients tested by multiple gene panels had pathogenic mutations identified in other breast cancer predisposition genes, namely, in *PALB2*, *CHEK2*, and *ATM*; however, many of these multigene panel tests also identified many variants of uncertain significance, where the classification is either uncertain or simply just not possible—and this information cannot safely be utilized clinically [19].

Indeed, this consideration is shared in recent literature and has been extended by Thompson et al. where the authors examined various genes that are common on hereditary breast cancer panels. They observed that the frequency of mutations in most breast cancer panel genes is quite low, or even, in most cases, similar to the frequency of mutations observed in cancer-free population controls. They concluded that panels have the potential to provide clinical misinformation and harm at the individual level if the data is not interpreted with extreme caution [14]. This lack of evidence for new genetic players is not limited to breast and ovarian cancer, but it also plagues many other genetic diseases and also afflicts a great number of other multigene panels [19].

Due to this lack of supportive data, international large-scale studies into the genes included in these multigene panels are absolutely critical to increase the utility of the information yielded and also to ensure that the new genetic information presented is both safe and useful in a clinical setting.

6. Conclusions

Keeping in-line with global regulation entities such as CAT, EGAPP, NHS UKGTN, and the ACCE backbone discussed above, the recent initiative eviQ provides a variety of guidelines for cancer genetics investigations. EviQ is part of the Cancer Institute NSW (New South Wales) and provides evidence-based information to support health professionals in the delivery of cancer treatments available at the time treatment decisions are being made. We can see from the eviQ's general practitioner referral guidelines for cancer genetics assessment that generally, even in oncology, genetics plays a large part in the diagnostics of disease [23]. We can also note that there must be a strong clinical suspicion of the diagnosis involving a genetic element (e.g., quite young, strong family history, hailing from a region with restricted gene flow, etc.) to warrant testing and for it to be useful.

The clinical utility of genetics is highly variable and dependent on the gene or disorder involved, but genomics medicine appears to be very good at revealing a diagnosis, and, at times, it can help explain why the phenotype is the way it is. In oncology, genetic testing plays an integral role in disease management by influencing treatment options or by being a major inclusion component for clinical trials. Where genetic testing is taking off and, perhaps, where its true potential lies is in its ability to offer predictive and preventative medicine, particularly for families, as opposed to adhering to a purely reactive approach that is typically employed in mainstream medicine.

Genomics is by no means irrelevant; it is revealing much about human disease and pathophysiology, but barring the current leaps and bounds observed in oncology, genomics medicine is still in an informational gathering phase—albeit, it is doing so at an alarming and unparalleled rate. It will take a great deal more data, analysis, and time before it will be considered true mainstream medicine, but there is no doubt that genomics is the future of medicine.

Conflict of interest

The authors declare no conflict of interest.

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The field of medical genetics and genomics has been constantly revolutionized by new breakthroughs, which bring more knowledge into the etiology and help improve the health care of individuals with either rare or common diseases. Nevertheless, as technologies evolve, novel challenges emerge, both technically and ethically, so they must be prudentially addressed. Among the myriad applications of genomics in medicine, this book depicts a glimpse of the advances achieved that have been leading us to the personalized/precision medicine era.

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