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Research Article

Preimplantation Genetic Testing and Cystic Fibrosis

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ABSTRACT

Cystic Fibrosis (CF) is one of the most common autosomal recessive disorders, as well as it is among the most common diseases diagnosed by prenatal and Preimplantation Genetic Testing (PGT) for monogenic conditions. The main focus of our research was to analyze the state of PGT for CF. The objective was to systematically review developments in the field of PGT from 2010 to 2020, giving an overview of currently available diagnostic tools, their efficiency and complications, their cost and perceptions of patients and doctors. The selection process included extensive search of databases like PubMed, DOAJ and Grey Literature (Google Scholar). A total of 700 papers were identified, of which 310 were eligible for title and abstract screening. After following specific inclusion criteria, a final number of 39 papers were included. The field of pre-implantation genetic testing is constantly evolving, with new technologies and approaches emerging fast. Studies have shown that PGT is nowadays used more frequently than prenatal testing. Because of the sheer number of studies and different existing approaches, it is becoming progressively more challenging to reach standardization. One of the main findings was that ethnicity is a major influence or obstacle. This is primarily due to populations becoming increasingly multiethnic, which makes it difficult to allocate a distinct ethnicity to one individual. Traditional ethnicity-based approaches could lead to individuals of non-traditional backgrounds to miss the opportunity of screening. Revision and reviews of available studies, comparison of outcomes and summarizing present problems could help achieve uniformity in testing.

Keywords: Pre-implantation genetic testing; Pre-implantation genetic diagnosis; Expanded carrier screening; Cystic fibrosis; Monogenic disorders

INTRODUCTION

Cystic Fibrosis is a severe autosomal recessive disorder characterized by the buildup of thick mucus in the lungs, pancreas, liver and other organs, which in turn leads to epithelial destruction. Both parents must carry one deleterious allele of the gene causing this condition. These couples have a one-in-four chance in every pregnancy to conceive an affected child. Even though CF is considered to be pan-ethnic, it is most prevalent in Caucasian populations, specifically individuals of northern European descent (1 in 26 carrier prevalence) and Ashkenazi Jews (1 in 25) [1]. It influences other ethnicities with varying frequency, being least common in individuals of Asian ancestry [1]. Fedick, et al. showed that clinical signs and symptoms of CF are primarily recurrent respiratory infections leading to progressive pulmonary failure, insufficiency of pancreatic exocrine function and Congenital Bilateral Absence or atrophy of the Vas Deferens (CBAVD) or obstructive azoospermia, which can lead to male infertility. Persistent infections can occur that lead to the production of large amounts of sputum, which initiate bronchiectasis and the destruction of the lungs. In 80%-95% of patients, the main cause of death is pulmonary failure, with the current median survival being approximately 37 years. Symptoms first occur in early childhood, although in some rare cases there is an adult onset [1]. In the 1990s, it was determined that the anion channel, Cystic Fibrosis Transmembrane Regulator (CFTR), manages the absorption or secretion of epithelial fluid and electrolytes [2]. If CFTR is

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reduced or absent, it can lead to functional changes, especially in the respiratory tract, causing the characteristic clinical symptoms of the disease, as stated by Stolz, et al. [3]. Until 2011, therapy options concentrated on management of abnormal CFTR functions, for example malabsorption, malnutrition, chronic respiratory disease and other associated disorders [4]. Since then, multidisciplinary, multisystem treatment options have emerged leading to increased survival and improvement in the quality of life. With earlier and better detection programs, patients can be identified before the onset of symptoms and there is the opportunity to focus on early gestational outcomes. CF is a single gene disorder that occurs due to mutation in the *CFTR* gene, located on chromosome 7 [5]. There are various DNA sequence variants that affect different molecular mechanisms and lead to CF. CF molecular genetic research has been focusing on the identification and classification of disease-causing CFTR mutations [4]. Welsh, et al. in 1993 determined that CFTR, a transmembrane protein, is made up of five domains. One domain has a regulator function, to control the activity of the channel. There are two domains that bind ATP (NBF1 and NBF2), that also exhibit regulatory functions. The final two domains are membrane-spanning (TM1-TM12) that make up the chloride channel pore [6]. There are over 2000 mutations in the Cystic Fibrosis Mutation Database (www.genet.sickkids.on.ca/cftr/app).With the help of databases of numerous variants for thousands of individuals new pathogenic mutations can be discovered, this paved the way for new diagnostic approaches [7]. There are five groups of CFTR mutations, characterized by their impact on gene function. According to Kerem et al. in 1989, c.1521_1523delCTT, p.Phe508del is the most commonly occurring CF mutation. It occurs in two thirds of all cases [8]. There are mutations that are present more frequently in certain populations, for example, the c.3846G>A, p.Trp1282X mutation is very prevalent in people of Ashkenazi Jewish descent, probably due to the founder effect [9]. Another example is c.3302T>A, p.Met1101Lys, a mutation occurring in the Hutterites of South Dakota [10]. The mutation incidence also varies within geographical regions, i.e., p.Phe508del has a frequency of 90% in Denmark, which decreases to 24% in Turkey [11]. The other third of alleles is considerably more heterogeneous, making up 10 to 20 less common pathogenic variants that ensue at a low frequency of 0.1%, together with many other rare or novel variants [12]. As sequencing is improving, many variants that are not pathogenic or have unknown significance (VUS) are discovered, which poses a new challenge of variant interpretation rather than detection [4]. Castellani, et al. suggested the classification of *CFTR* variants based on their clinical phenotypes [13]. Group (A) consist of variants that lost their function and cause the disorder when they occur simultaneously, (B) are variants with some remaining CFTR function, and therefore show more mild phenotypes, (C) make up variants that have no clinical consequences; and (D) includes VUS. In the last 30 years, there has been a tremendous improvement in the screening and diagnosis of CF. In 1990, PGD was described for the first time, with the aim of female embryo collection for X-linked conditions [14]. Nowadays it represents an alternative to prenatal diagnosis, helping high risk couples to deliver a healthy child and to avoid pregnancy terminations [15]. CF was the first single gene condition to be examined in PGD research [16]. Improvement in diagnostic technologies increased the number of fertile couples undergoing pre-implantation genetic testing at IVF centers. PGD is useful in routine testing of common syndromes, like CF, fragile X syndrome and spinal muscular atrophy. It can also be applied in expanded carrier screening panels, inherited cancer genes, adult-onset neuromuscular disorders and Human Leukocyte Antigen (HLA) matching [17]. Due to the incidence of CF and the high heterozygosity in Caucasian individuals, research of CFTR nowadays signifies one of the most common routine genetic analysis worldwide, whether to diagnose CF or CFTR-related disorders, or to suggest carrier testing, prenatal diagnosis or PGD [12]. The method is centered on the genetic assessment of embryonic cells taken from an embryo biopsy by *In-Vitro* Fertilization (IVF) methods. Only embryos that are determined to not have a disease are transferable for pregnancy [18]. Even before the transfer, PGT can be used to determine if the embryo is euploid and if mosaicism is present. Next Generation Sequencing (NGS) is believed to be the best approach, together with trophectoderm and blastocyst stage biopsy [19]. Currently available methods for mutation screening and gene scanning include Sanger sequencing, high resolution melting curve analysis and quantitative fluorescent melting curve analysis among others. NGS platforms commonly used in diagnostic laboratories include illumina, ion torrent and SOLIDTM System, which mainly differ in the instruments needed to perform each method. [12]. One of these approaches described in the systematic literature review include CFTR_MASTR Dx, which works on Illumina [20]. The main goal of carrier screening is to detect couples, who are at risk of transmitting a genetic condition, and provide the necessary genetic counseling, explaining the reproductive risk and management alternatives. CF is the most common indication for carrier screening and shows the importance of *CFTR* variant interpretation regarding pathogenicity [7]. Factors that should be considered when planning an ECS program are consanguinity, disease severity and variant pathogenicity. Regarding variant pathogenicity, ECS is facing challenges in interpretation and disclosure of results.

There are two approaches, a conservative method to only include well-established variants described by the ClinVar database, or a more liberal approach, which includes considering variants that are expected to be disease-causing by computational evaluation [7]. VUS comprise non-synonymous variants that are believed to be disease-causing but have not been seen in patients. Most VUS are found to not be pathogenic over time. The ACOG guidelines presented in 2017 state that ECS should ideally be performed before pregnancy. Individuals should be free to choose if they want to undergo testing, but all couples should be offered ECS for severe disorders, such as CF. All couples that choose testing, should be recommended genetic counselling for any further questions [7]. Recent developments have led to fast and affordable Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS). One of the most common approaches for PGD consists of whole genome amplification, with subsequent PCR for Short Tandem Repeats (STRs). STRs are characterized by great polymorphism and can be found anywhere in the genome, which makes them a good

method for linkage analysis, a foundation of PGD. This method is however limited by the risk of recombination, which can lead to misdiagnosis [21]. Another disadvantage is the possibility of allele dropout. PGD can also be done by utilizing SNPs for linkage. Compared to STRs they tend to be more densely located in the genome. Laboratories usually perform a SNP microarray, also known as karyomapping [22]. ECS can be performed by using WGS, which offers the benefits for pathogenic variant detection in non-coding regions. Moreover, it has the advantage of a more standardized analysis of the genome and improved evaluation of Copy Number Variants (CNVs), which also includes autosomal recessive genes [7]. Multiple studies indicated that embryo aneuploidy is the main cause for the failure of IVF procedures. This led to an increase in Preimplantation Genetic Testing for aneuploidy (PGT-A) [19]. PGT-A determines if the biopsied embryonal cells are euploid before moving on with transfer into the uterus [15]. PGT for Monogenic diseases (PGT-M) is suggested for partners who have a higher probability of transferring a genetic disorder. Sometimes chromosomal aneuploidies can occur on chromosomes that are not tested in PGT-M. This highlights the importance of screening for both aneuploidies and monogenic disorders. The first successful attempt of a double factor analysis was performed by Obradors, et al. in 2008 [23], with the goal to select an euploid embryo without CF mutations for implantation. More studies and improvements will be discussed in the following pages of this systematic review.

MATERIALS AND METHODS

The systemic review was conducted using the Cochrane collaboration protocol, followed by recommendations from Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement [24,25]. In this systematic review, the following databases were searched: PubMed, DOAJ and Grey literature (Google Scholar), to identify all published primary research studies in English. The search was restricted to human studies. The study time frame ranged from January 2015 to January 2021, however the beginning date was later moved to January 2010, due to a lack of relevant recent papers. All titles and abstracts were checked for inclusion by applying following inclusion criteria to determine if the abstract warranted further investigations:

- Primary research studies, that were full papers and original work, were included.
- Only studies written in English were added.
- Papers from the last 11 years were included.
- Any paper evaluating PGT for cystic fibrosis was included, with no restrictions regarding the country, patient age, race or gender.

For papers that fulfilled the criteria, full-text screening according to the research questions and data extraction was performed (Figure 1). The search strategies and terminology are available in the Supplements.

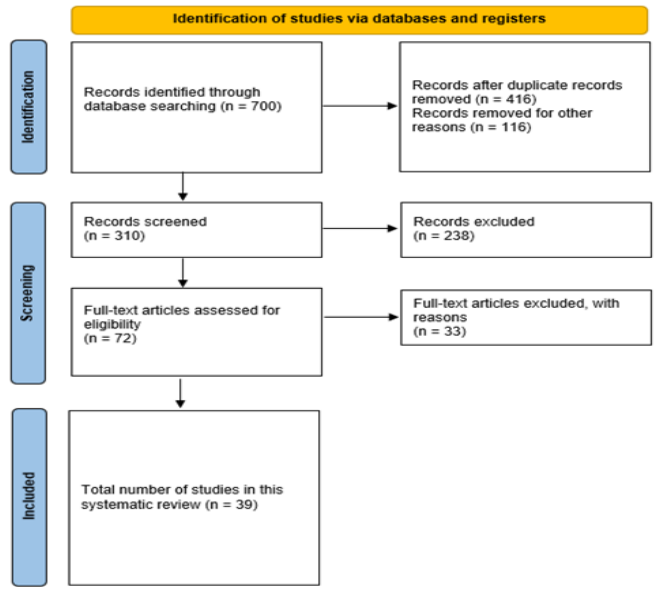

Figure 1: Schematic representation of the flow of information during the different phases of the systematic review (PRISMA 2020 flow diagram).

A total of 310 studies were used for title and abstract screening, of which 238 studies, that did not fulfill the criteria and aims of this review, were excluded. After assessing the full-text articles, 39 papers have been included in this systematic literature review. The presented studies focus on different screening methods and approaches on how to diagnose cystic fibrosis before implantation, usually in correlation to assisted reproductive technologies and *in-vitro* fertilization. Throughout the data extraction process, the studies were grouped into five areas, with some covering several fields. The highest number of articles were about methodology (n=21) and decision-making (n=10), other articles included outcomes and rates of PGD (n=4), cost-analyses (n=3) and external quality assessment (n=1). Detailed overviews of the studies used in this review are presented in the Supplements.

RESULTS

The findings show that pre-implantation genetic screening is carried out mostly in the form of Expanded Carrier Screening (ECS), Next Generation Sequencing (NGS) or dual screening approaches. Six out of 39 studies (15.38%) discussed ECS and NGS. In 2015, Franasiak, et al. compared three different panels, Inheritest, Counsyl 1.0 and 2.0 to understand how ECS affects clinical decision making. Of the 3738 couples tested, 1666 (25.1%) were determined to be carriers of at least one condition. Furthermore, it was determined that the number of couples needed to test to detect one couple requiring PGD is approximately 450. Three discovered cases of cystic fibrosis were already known before ECS, resulting in de novo findings of 1 in 748 cases in total [26]. Treff, et al. managed to establish an NGS-based method which was equally successful as two other, independent conventional PGD methods and showed 100%

reliability. [27]. In 2019, Chamayou, et al. universal approach to PGT was proposed using NGS for all cystic fibrosis gene mutations. PGT was carried out on 109 embryos, while screening included 15 different mutations. The results showed that PGT-CF was successful in 92.7%, while PGT-A had a slightly higher number of 95.3%. It is also important to note, that 81.3% of embryos that underwent testing and transfer, were able to be implanted successfully [28]. Five studies (12.8%) focused on a dual screening approach. The main study methods used for double factor analysis and outcomes are presented in Table 1.

Study	Study method	Study outcome
Artini et al.	Testing was done in Italian infertile individuals undertaking Intra Uterine Insemination (IUI) or *In-Vitro* Fertilization (IVF) by means of Intracytoplasmic Sperm Injection (ICSI). Karyotype and CFTR analysis were performed. (n=616)	In individuals undergoing IVF-ICSI 59 aberrant karyotypes were found-27 mutations in women and 32 in men. There was no couple with abnormal karyotypes in both individuals. For IUI, only one aberration was found in one woman.28 of the 420 tested couples carried CFTR mutations (6.66%). After analysis of results, 20 *CFTR* variants were found in IVF-ICSI patients, while 8 were found in IUI couples. There was no case where both partners were affected.
Daina et al.	Coriell cell lines carrying a CF mutation were used. Both monogenic (direct/indirect mutation analysis using tandem repeats) and cytogenic analysis (shortened mCGH protocol) were performed using the same WGA product. After completion of double-factor diagnosis (approx. 30 h after biopsy), the results were sent to IVF centers, which disclosed the genetic results and embryo development quality. If these results were compatible, embryo transfer was performed on the fifth day. (n=100)	WGA was successful in 59 out of 62 embryos. In the amplified embryo, monogenic diagnosis was carried out in 74.6%, and cytogenetic diagnosis in 98.3% Double diagnosis results were achieved in 43 out of 59 (72.9%). Of 29 embryos, who did not carry a monogenic disorder, 55.2% demonstrated chromosomal changes (such as aneuploidies, segmental imbalances, or both). Half of blastomeres presented with aneuploidy.
Rechitsky et al.	24-chromosome aneuploidy testing and PGD for monogenic disorder or HLA typing in the same biopsy sample was performed. 24-AT testing was microarray-based using a BlueGnome platform for aCGH. To determine the ADO rate caused by WGO, two types of mutations were used: *CFTR* and beta-globin gene mutation and compared to ADO rates in single cell analysis without WGA. (n=238)	The number of transferable embryos equaled 25.1% in blastomere and 42% in blastocyst biopsies. There was a total of 68.4% unaffected pregnancies and 149 healthy, HLA-matched children were born. The results differed from the 2,064 PGD cycles without simultaneous 24-AT testing, as there were improved pregnancy and spontaneous abortion reduction rates. ADO was highest after blastomere WGA and made up 27.7% for CFTR. The lowest ADO could be achieved for trophectoderm cells.
Goldman et al.	For PGD, linkage analysis by short tandem repeats was used, along with multiplex PCR along and direct mutation testing. aCGH was used to test for aneuploidy. SGD testing used the same WGA DNA. (n=57)	In the dual screening group, most embryos were not transferable because of aneuploidy. There were no substantial differences among the two groups. The percentages of blastocysts affected by SGD were similar for both groups (37% SGD+aCGH and 32.8% SGD-alone).
Zimmerman et al.	The study created a unique protocol for PGD of trophectoderm biopsies employing quantitative PCR (qPCR). Cell lines with already established genotypes were utilized. The results were matched to SGD reference laboratories. Testing was done on Coriell cell lines.	Fibroblast cell testing resulted in an ADO rate of 1.64%. When testing multiple cells, the rate decreased to 0.02%. Amplification failure equaled to 1.38% in total. Of 152 embryos, 17 cases were screened using qPCR. Analysis could be made in 100% and there was no ADO or amplification failure. Compared to reference laboratories, outcomes were similar to>99%. The pregnancy rate was 82%.

Table 1: Main methods and outcomes of dual screening studies.

In relation to CF, we have found that by far the most frequent mutation is c.1521_15 23delCTT (p.F508del), which was mentioned by 17 out of 39 papers (44%). The variant c. 1624G>T (p.Gly542Ter) was mentioned in 9 papers (23%). Thereafter, variants c.3846G>A (p.Trp1282Ter) and c.3909C>G (p.Asn1303Lys) were mentioned in 7 out of 39 papers (18%). All these mutations are classified as pathogenic and have a high frequency (Figure 2).

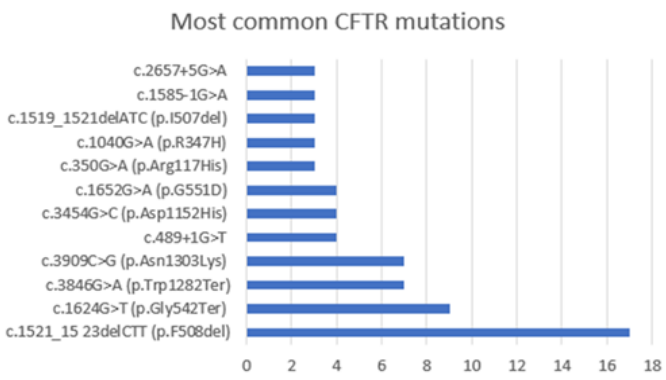

Figure 2: Most common CFTR mutations and variants, based on the number of studies that tested for these specific mutations.

However, findings can be complicated by misinterpretations, usually due to benign variants or variants of unknown significance. Next figure shows that most included variants tend to carry an "increased risk" and pathogenicity (Figure 3), only one variant of varying consequences and no "low risk" variants were commonly mentioned by multiple papers.

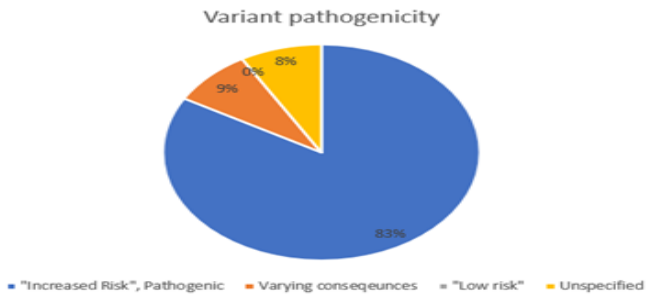

Figure 3: Variant pathogenicity, as described by the included studies. **Note:** Increased risk, pathogenic, Varying consequences, Low risk, unspecified.

Three cost-benefit analyses of PGT for CF were included to establish the net benefit of this approach over natural conception in couples that are CF carriers. Davis, et al. showed in 2010, that the net benefit of PGD over Natural Conception (NC) was highest in women under the age of 35 and was less as maternal age increased [29]. Tur-Kaspa et al. evaluated the use of PGD to avoid CF births, by assessing the cost of IVF-PGD for CF carrier couples in contrast to medical costs of treating new CF patients.

The results showed that medical expenses for management of a CF patient equaled to $63,127. Treatment of 4000 CF carriers with IVF-PGD in one year leads to 3751 healthy babies, with a cost of $57,467 per child. Savings would equal to $2.3 million per patient and $2.2 billion for all new CF patients annually in lifetime treatment costs [30]. In 2012, Norman, et al. wanted to determine the cost of carrier screening for CF from pregnancy to pregnancy. They showed that initial pregnancy prenatal screening decreased CF births by 53%. Without screening, 40/100,000 initial pregnancies and 30/100,000 subsequent pregnancies in Australia lead to the birth of a child affected by CF. The study moreover proved that initial pregnancy screening costs were higher than in populations where no screening was undertaken (A$16.6 million/100,000 births to A$13.4 million, respectively). The incremental cost per CF in the first pregnancy equaled to A $150,000. However, the cost decreased for any subsequent pregnancy [31]. Additionally, it was shown that testing alone is not enough to guide couples during the reproductive decision-making. Patients and doctors' behaviors and opinions during the testing process are also evaluated and reflected. A total of nine studies (23%) focused on the decision-making process or individual's attitudes towards CF screening and the use of PGD. The findings showed mainly positive views on population CF carrier screening. There was a high knowledge of carrier status and risk of transmitting CF, especially among carriers. The best screening time was determined to be before pregnancy, while most individuals also valued the free decision whether to take a carrier test or not. The participants of three studies planned to use PGD or did so in the previous three months. 13 couples planned not to change their reproductive choices. Most carriers (94%) would recommend screening to their family members and friends, while 41% of non-carriers would do so. However, not many family members reported to undergo testing. When asked who should provide PGD, the participants mostly stated gynecologists and clinical geneticists, after which come general practitioners and suppliers of preconception consultations. Hershberger et al. stated that individuals with a high-risk ethnic background usually undergo genetic screening to understand their genetic risk before conception [32]. Reasons for declining participation included mostly time limitations, no interest, not wanting to have the knowledge and possible trigger of concern and anxiety. Focusing on ECS, 31% of participants would take part in expanded screening themselves. 55% believed ECS should be suggested to anyone planning to become pregnant. Some concerns were raised about the prospective negative outcomes of a population-wide CF carrier screen. Also, some participants believed that screening could be the reason for increased terminations of pregnancy. Gilmore, et al. could not find any association between concerns with privacy or discrimination and ethnic background [33]. Morrow et al. aimed to determine obstetrician understands of PGD in 2016. A total of 398 physicians took the survey, of which 26 were excluded as they were not practicing doctors. The results of the study can be found in Figure 4.

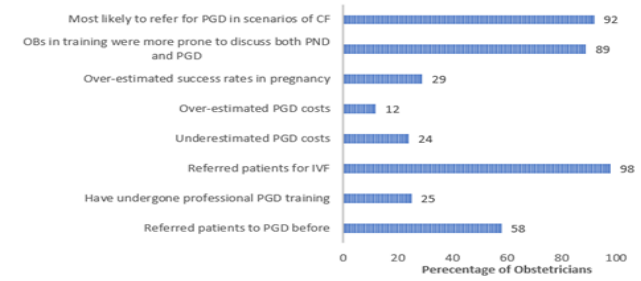

Figure 4: Morrow et al. obstetricians' understanding of PGD.

The main findings can be found in Table 2, while Table 3 discusses specific details of success rates. Figure 5 describes the main findings in the study performed by Olive, et al. in 2011. It describes the rate of occurrence of complications and defines what type of complication occurred most. Table 4 discusses the results of two studies correlating prenatal diagnostics to preimplantation genetic testing. In addition, it was determined that the main challenge for PGT is the creation of an ideal, standardized screening panel. While analyzing PGT methods as well as CF screening, one of the main findings was that ethnicity is a major influence and or obstacle. When looking at the ethnic distribution of ECS studies, out of 6 studies, three did not specify the ethnic distribution. The other findings are summarized in Table 5.

Study	Main findings
Olive et al.	The main finding is the low birth abnormality rate of 1.42%. Yet, 22.9% of infants needed special treatment, and health problems after birth occurred in 22.3%.
VanWort et al.	No link could be found between female CF carrier status and response and outcomes to IVF.
Sharpe et al.	The live birth with PGD is higher than in non-PGD, although the difference is not significant.
Poulton et al.	The study showed that PGT-M is used more frequently than PNDx nowadays.
Priner et al.	The study indicated that repeated biopsy was useful in cases where genetic diagnosis was not able in the first run.

Table 2: Main findings of studies discussing PGD outcomes and or success rates.

Study	Success rates of PGD
Olive et al.	Out of 494 children born in PGD cycles, 76.7% babies did not have any health issues, while 129 babies (26%) had neonatal complications.
VanWort et al.	Patients with the R117H mutation, which was found in 14 cycles, showed a significant decrease of retrieved oocytes and number of 2 pronuclei embryos. Patients carrying the W1282X mutation, found in 17 cycles, presented with increased numbers of retrieved oocytes and 2PN embryos. For DF508 (n=84 cycles) a substantial rise was present in 2PN embryos.
Sharpe et al.	Every fourth embryo is not transferable after PGD and in 94% of cases this was due to a failure to survive. There were 55% negative outcomes, 34% had a detectable fetal heart rate, 6% had a biochemical pregnancy, 4% miscarried and<1% was an ectopic pregnancy. Couples did not conceive in 949 cases.
Priner, et al.	Genetic diagnosis was able in 82.7% cases where repeated biopsy on the same embryo was performed. The rate was higher in Polar body Biopsy (PB) followed by cleavage-stage biopsy (BB), rather than BB followed by trophectoderm biopsy.

Table 3: Success rates of PGD.

The outcomes and success rates of testing were also included to describe any associated birth anomalies and complications. Five studies (12.8%) were included, of which four were retrospective and one study evaluated the use of repeated embryo biopsy for PGD.

Study	Findings
Sharpe et al.	The use of PGD has increased 127 times from 1991 to 2012, and there was a 360% increase from 2004 to 2012.
Poulton et al.	While prenatal testing (PNDx) was funded by the government and provided in public hospitals, pre-implantation testing for monogenic disorders (PGT-M) was firstly offered under research protocol and was available from 2002 in clinical care, and not funded by the government. After the year 2000, an annual PNDx rate for monogenic diseases was between 1.3 to 2.2 per 1,000 births. PNDx was performed in 72% for conditions that affect physical capability, while it was rare for adult-onset disorders (3%). Since the development of PGT-M, its use has risen, and it is similar to that of PNDx. However, it is used more commonly in adult-onset disorders. The most widespread indication for PNDx and PGT-M was cystic fibrosis.

Table 4: Comparison of PGD to other diagnostic methods.

Study	Ethnic distribution	Sample size
Treff et al.	Not specified	n=12
Franasiak et al.	51.8% Caucasian, 14.9% Asian, 8.1% Hispanic, 6.6% African American, 0.1% American Indian, 2.3% "other", 16.2% chose not to report	n=6.642
Behar et al.	53.3% Jewish or mixed Jewish/non-Jewish ancestry, 48.7% were Arabs, Druze or mixed Arab/Caucasian origin. Arab CF patients were sub stratified as Muslims, Christians or Bedouin	n=176
Capalbo et al.	Not specified	n=14,125
Chamayou et al.	Not specified	n=1052
Hernandez-Nieto et al.	80.5% Latino, 8.6% European, 8.2% Jewish, 2.4% Middle Eastern, and 0.3% others	n=805

Table 5: Ethnic distribution of ECS studies.

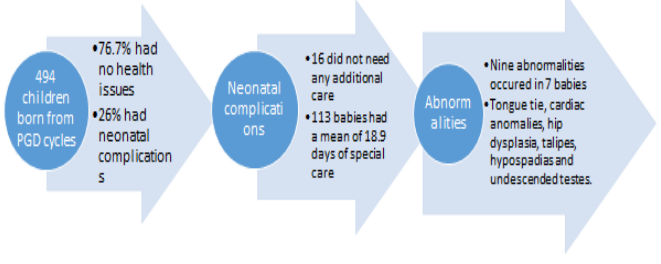

Figure 5: Olive et al. : Findings on neonatal complications.

Hernandez-Nieto et al. analyzed the carrier status according to the different ethnicities within their Mexican cohort and found that patients who identified as Jewish were carriers of at least one condition in 65.7%. This is followed by the Middle Eastern population, Latino and European ethnicities. Cystic fibrosis was found in 22 cases, which equals to a prevalence of 3.44% [34].

Behar, et al. conducted research, testing an Israeli preconception screening program that focused on 22 ethnically targeted mutations. They managed to identify 54 mutations, of which only 16 overlapped with the current Israeli screening program. Of those, 53.7% were already stated in the CFTR2 database and only 7.4% were novel. Prenatal diagnosis of 30.8% of cases could have been reached by inclusion of all Israeli panel variants [35]. Capalbo et al. stated that one of their main limitations during research was the lack of ethnic diversity in their population [36]. Franasiak et al. found that of the 1,666 positive tests, 65.8% were Caucasian, 8.4% Asian, 5.5% African American, 1.6% "other", 0.1% American Indian and 18.7% did not report their ethnic background [26]. Zeevi et al. compared three reference panels to determine the efficiency of ethnicity-matching to panel size.

They divided the cohort into three subgroups: Full Ashkenazi (FA; n=16), mixed Ashkenazi (MA; n=23) and non-Ashkenazi (NA; n=15). The FA subgroup showed the lowest phasing accuracy, although the data set was the largest panel. Improved results were found in smaller ethnicity-matched reference panels. It could be proved that ethnicity matching and large reference panels can significantly increase the success of population-based haplotype phasing. Twelve individuals were carriers of CFTR founder mutations. In eight cases the W1282X variant was present, of those five individuals were of MA ethnicity and three FA. In four the delF508 variant was found, all individuals were of FA descent. However, the study presented an unpredictable phasing accuracy, especially for carriers of MA background. On the other hand, high accuracy was found for FA phasing [37].

DISCUSSION

Expanded carrier screening

The field of pre-implantation genetic diagnosis is advancing at a fast pace, as the methods for genetic analysis are changing and enabling new approaches towards identifying and screening individuals or couples that carry a risk for spreading genetic conditions to their children. The goal is to provide couples with the necessary information to understand their risk and help them with finding the right reproductive choice. As shown in the results section, PGS is mostly performed by ECS; NGS or dual screening approaches. The American College of Obstetricians and Gynecologists (ACOG) issued a statement that determined that ethnicity-based, pan-ethnic, and expanded carrier screening all can be used as acceptable methods for carrier screening before and during pregnancy [38]. Introducing an expanded disease panel for all populations is necessary since Mendelian diseases are the reason for 20% of infant deaths and 18% of infant hospitalizations in the US [39]. There are also various public health and individual advantages, such as better accessibility and improved prevention and management, decreased time and cost of diagnosis, improved quality of life and reduction of unnecessary therapy. Studies have shown that population screening can decrease the incidence of disease of interest [40] and performing large-scale ECS could have a significant influence on mortality and morbidity. ECS usually requires further tests and genetic counseling, more so than in traditional ethnicity-based approaches. This is due to the possibility of screening multiple disorders, and therefore the increased identification of carriers. Several studies have found the carrier identification rate by using ECS panels to be approximately 24% to well over 50% [41,42]. This leads to an increase in time and cost spent on downstream genetic testing, such as partner screening, PGD and other approaches. This has also been supported by study findings of Franasiak, et al. [26]. At-risk couples, with potentially highly disabling disease, demand clinical counseling for reproductive options, with more personalized diagnostic and therapeutic management [32]. More universal counseling could lead to better patient responsiveness to different disease types on respective existing panels [38]. Disadvantages of ECS include the lack of uniformity and standardization for test panels and screening methods, as well as no regulation of companies. The inconsistency of laboratory methods leads to the inclusion of conditions that are not recommended by common guidelines. Capalbo, et al. showed in their study that present expanded screening technologies are not able to find all variants important for pre-implantation carrier screening, including triple repeat disorders and genomic regions with high homology [36]. There is the need to increase ECS sensitivity and improve the PCS offer. Further research should be conducted to evaluate detection of genetic mutations and disease variants, as well as to determine the success of different panels on identifying carriers. Additionally, the development of standardized "ideal" genetic disease panels is necessary. Modifications should be possible for individual preferences and values.

Next generation sequencing

Bell et al. [43] conducted a study of 448 recessive diseases analyzed by NGS and confirmed the high analytic sensitivity and specificity of this method. This was also validated by Treff et al. who showed that NGS was able to diagnose conditions as well as those with PGD [27]. NGS was found to be superior to other methods, when used as a universal strategy to diagnose CF mutations, because various polymorphisms can be verified in only one cycle, while at the same time PGT-A and-CF can be carried out [28]. However, Lim et al. [44] discovered that present genotyping panels do not show great success for minorities, especially South and East Asian populations. While pan-ethnic screening populations for CF already exist, the most common mutations used in these panels are chosen based on their frequencies in Caucasians. This does not represent all ethnicities, and therefore is not an ideal population-based method. NGS is a very complex gene assay and it presents with difficulties when it comes to CF, a condition with complex genetics and genotype-phenotype relationships. This makes it difficult to determine uniform mutation search strategies. NGS can be applied well for the *CFTR* gene, because of its size and the great number of known mutations. However, findings can be complicated by the detection of benign variants or VUS. These misinterpretations can lead to a noteworthy overestimate of present pathogenic variants.

Dual screening-chromosomal abnormalities

Most couples who choose to undergo PGD require assisted reproductive technologies for conceiving. The indications for IVF, including increased maternal age, infertility or recurrent pregnancy loss, all can be connected to chromosomal abnormalities. With the increased prevalence of aneuploidies, determined also in younger patients [45], the possibility to couple aneuploidy and monogenic disorder screening poses a groundbreaking technological improvement. The included studies show the high success rates and accurate diagnostic performance of this approach, indicating a substantial consequence on reproductive medicine and genetic testing.

CFTR mutations and variants

There has been a continuous identification of new CF variants for over 30 years. At this time, over 2,000 variants have been listed in the CFMDB; yet only 442 can be found in the CFTR2

database, of which 360 are believed to be disease-causing [46]. Disease severity depends on the detrimental effects that mutations have on a gene. The past efforts of CF carrier screening and the wide range of phenotypic presentations make it difficult to classify each mutation [47]. As use of NGS has increased over the recent years, the next step is to aim to understand about already known variants. Transparent techniques of variant categorization are essential, especially as the results of these carrier screens lead to important personal choices [48]. Behar et al. [49] mentioned that it is difficult to establish a general preconception carrier screening program in heterogeneous populations, where there are numerous different mutations and many individuals do not have a molecular diagnosis. Finding additional CF-causing mutations can aid in forming a pan-population CF preconception screening panel. Future studies should focus on the determination of pathogenicity of mutations by aiming for functional proof, thereby clarifying if pathogenic effects have been attributed mistakenly. Considering all mutations discovered in the study, an estimate was made that an Israeli pan-population detection rate of 85% could be achieved. Another point for future studies is sequencing for spouses of carriers, for which more empirical data is needed. There have been some issues regarding Variants of Unknown Significance (VUS) that make the screening and counseling process difficult. Commonly, many laboratories do not include VUS in the report for patient counseling [50]. The ACMG has issued standard criteria for analysis of genetic variants [51], but the approach might still be complicated by rare conditions and unclear outcomes. In 2018, Punj et al. were able to show that genome sequencing increased clinical sensitivity for detection of pathogenic variants, compared to targeted mutation screening. Most of the found variants were disease-causing. The use of GS with a great number of gene/disorder pairs led to higher sensitivity for diagnosis of clinically important variants [33]. Variant and mutation classification have proven to be one of the main tasks in the diagnosis of CF. Mainly due to the ongoing detection of new variants, the occurrence of VUS and the high heterozygosity of the screened populations. This makes it difficult to initiate a pan-population preconception screening program and offer a clear mutation panel. Cooperation between major laboratories and IVF clinics, updated databases and clear guidelines and uniformity of the process, could all help to make a more standardized and clear approach to the classification of *CFTR* variants.

Decision making/attitudes towards CF Screening

The studies have determined that there are generally positive views towards PGS, as shown by Maxwell et al. in 2010 or Ioannou et al.. Patients were primarily influenced by the severity of the disorder, as well as by their doctor's recommendations. It has been shown that when doctors have sufficient training, they are more likely to discuss and offer genetic testing to their patients and moreover refer their patients correctly, especially in cases of severe conditions such as CF and spinal muscular atrophy. These findings indicate the need to make training programs for doctors encountering at-risk patients and to teach them the correct approach to increase their patients' understanding of PGD, genetic disorders and inheritance patterns. In 2016, Morrow et al. aimed to determine obstetricians' understanding of PGD and to find obstacles when referring patients [52]. The study showed that most doctors lacked professional training about PGD but dealt with many patients undergoing IVF. They were unsure about the cost of PGD, as well as the success rates in pregnancy. The doctors found their patients' financial situation and limited access to PGD to be major obstacles. Others included religious reasons, inability to conceive naturally and marriage status. Obstetricians with training were more prone to consider both PND and PGD. In the more difficult scenarios, they were able to choose referral correctly. Most obstetricians referred correctly for PGD in the settings of spinal muscular atrophy and CF. The outcome of this study indicates the necessity of better training of doctors to offer better information to couples. Janssens et al. started a study to determine the opinions of CF patients and parents towards carrier screening and other reproductive concerns [53]. A structured questionnaire was used, to prove that nearly all partakers were supportive of population-based carrier screening. The most often selected reproductive method was PGD, after that came spontaneous pregnancy coupled with prenatal diagnosis. However, it should be mentioned that CF patients suffer from fertility complications and therefore are more likely to choose reproductive counseling, which explains their preference for PGD.

Cost-benefit analysis

Three cost benefit analyses of PGT for CF were able to prove the net benefit of this approach compared to natural conception in those couples, who were carriers of cystic fibrosis. Cost-benefit analyses are important before initiating any new medical technology or treatment. In 2010 in the United States of America, Davis et al. [29] presented a cost-benefit assessment of PGD for couples, who are CF carriers and correlated those results to couples choosing Natural Conception (NC). Besides a better net benefit for PGD, there are a number of other advantages: Substantial reduction in abortions and the enhanced possibility of experiencing a live birth. The results of this study indicated significant net benefits of PGD over NC, which decrease with increasing maternal age. Another cost-benefit analysis was performed by Tur-Kaspa et al. [30] in 2010. The purpose of this analysis was to evaluate the cost-benefit for a national IVF-50 PGD program to prevent CF by linking the price of IVF-PGD for all +/+CF couples to the direct medical expenses conserved by avoiding the management of novel CF patients. Savings would result in $2.3 million for one patient and $2.2 billion for novel CF patients annually in lifetime treatment costs. The study proved that implementation of a nationwide IVF-PGD plan is very profitable and as part of preventive medicine, would prevent birth of children with devastating genetic conditions. Norman, et al. [31] performed an Australian cost-effectiveness study in 2012. They wanted to determine the cost of carrier screening for CF from pregnancy to pregnancy. The results of this study indicate that a national carrier screening program for CF would be beneficial, as it would decrease disease incidence with an acceptable, or even potentially negative, expense. In summary, there is a better net benefit of PGD over natural conception in couples that are CF

carriers. PGD is further associated with other advantages, such as the increased rate of life births, as well as the decreased incidence of abortions, and thereby not only reducing medical costs but also the major psychological and emotional burden on couples. However, these findings are diminished with increasing maternal age. Overall, these studies prove that the implementation of a nationwide IVF-PGD program would be highly cost effective and would be a major step towards preventive medicine.

Outcomes and success rates of PGD

The studies making up this section have shown that PGD is associated with low birth anomalies and complications. However, for approximately 20% of cases there is an occurrence of health problems after birth and the need for special neonatal care. The study of Olive, et al. showed that completed parental questionnaires offer useful data on long term health and outcomes of PGD/PGS [54]. Overall, usage of PGD increases live birth rates, especially in younger women, and reduces the incidence of congenital abnormalities. As there is more development in this field, more couples will experience benefits from PGD with best possible live birth rates and full-term healthy offspring [55]. It is crucial to mention that being a CF carrier does not impact reproductive outcomes for IVF; with no changes in implantation rates or pregnancy outcomes [56]. For a long time, prenatal carrier testing started with the evaluation of family history followed by Prenatal Diagnosis (PNDx) and it was the routine method of examination. Recently, there have been many technical improvements in this field, such as preimplantation genetic diagnosis, screening of carriers, noninvasive prenatal diagnosis and never therapy options performed at a postnatal stage. It has been discovered that PGT-M is used more frequently than PNDx nowadays. Compared to prenatal genetic diagnosis, which for years had been the routine approach, pre-implantation genetic testing is not funded by the government and individuals choosing this option have visited private IVF clinics at their own expense. Nevertheless, due to major technical updates PGT is used more frequently nowadays [57]. Continuing examination of PNDx and PGT-M for SGDs will be essential for observing the influence of modern technologies in reproductive medicine.

The creation of a standardized screening panel

The most difficult part of PGT is to form an ideal, standardized screening panel. Ethnicity poses one of the main challenges in this process. The ACOG observed the ethnic disproportion of CF frequency. There are existing guidelines for screening for individuals of Caucasian or Ashkenazi Jewish descent [5]. However, these recommendations were updated in 2005 to rationalize pan-ethnic screening as it is becoming gradually more problematic to allocate a particular ethnic background to persons [58]. In recent years, the number of individuals with mixed descent has increased and it is more challenging to determine a definitive ethnicity. At-risk couples could be missed, the residual risk is difficult to determine, and the accuracy of counseling is diminished. Albeit it is acknowledged that gene disorders are more common in specific populations, it does not necessarily have to be limited to this ethnicity. This could lead to individuals of non-traditional ethnicities to miss the opportunity of screening. As mentioned before, there are low detection rates and misdiagnosis in mixed-ethnicity populations, which puts a great importance on NGS for correct diagnosis of these groups. Most carriers can be missed if the standard panel, recommended by ACOG, would be used.

CONCLUSION

NGS made it possible to expand the panel and screen for more variants, which allowed revealing population-specific pathogenic variants. Different from mutation panels, NGS can be used to analyze variants within the whole target sequence. But even though it can reach a very great diagnostic yield, it is associated with significant time and work effort, and standardization is challenging. Behar, et al. found in their study, that pan-ethnic expanded panels in combination with well-curated dataset of CF-causing mutations, can be used to achieve higher detection rates. Another benefit of this approach is the avoidance of complications associated with whole gene sequencing. Hernandez-Nieto et al. used a panel targeting multiple ethnicities by screening for multiple diseases and using full gene sequencing, instead of focusing on one specific ethnicity. It has been established that using ethnicity-based approaches is of less value for ECS. They propose that all individuals should be offered expanded disease screening irrespective of their ethnic background. The increased interest in universal screening, together with lower costs associated with screening for multiple diseases at the same time, has changed both ECS approaches and newborn screening.

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COMPETING INTERESTS

The author(s) declare no competing interests. The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

MK performed the systematic analysis, drafted the manuscript and provided critical revisions of the manuscript. EE provided critical revisions of the manuscript. AH designed and led the research, participated in the drafting of the manuscript and provided critical revisions of the manuscript. All authors provided critical feedback on the manuscript and approved the final manuscript.

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