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The Spectrum of Preconception Carrier Screening: Review of Targeted Gene Screening to Comprehensive Approaches

Mini Review

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Author Details

Unsal E[1,2], Aktuna S[3] and Özer L[3]*

[1]Department of Obstetrics Gynecology and Reproductive Sciences, Yale School of Medicine, USA

[2] Yale Fertility Center, USA

[3]Mikrogen Genetic Diagnosis Center, Türkiye

*Corresponding author

Evrim Unsal PhD, Associate Research Scientist, Division of Reproductive Endocrinology & Infertility, Department of Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine, Orange- 06477, USA

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Abstract

It is estimated that there are more than 1800 inherited rare diseases, and their prevalence differs based on geographic locations, influenced by genetic diversity, environmental factors, societal conditions. Preconception Carrier Screening has a crucial role to play in the prevention of these rare diseases in future generations. Reproductive decision-making is the established measure of the clinical utility of population-based screening that guides couples to make informed reproductive decisions.

Keywords: Expanded Carrier Screening, Preconception Carrier Screening, Whole Exome Sequencing, Whole Genome Sequencing, Reproduction, Consanguineous Marriage, Rare Diseases, Preimplantation Genetic Test

Abbreviations: RDs: Rare diseases; NGS: Next-Generation Sequencing; ECS: Expanded Carrier Screening; WES: Whole Exome Sequencing; ACMG: American College of Medical Genetics; VUS: Variant of Unsignificance; CF: Cystic Fibrosis; SMA: Spinal Muscular Atrophy; SNVs: Single Nucleotide Variants; CNVs: Copy Number Variations

Background

Rare diseases (RDs) encompass approximately 7,000 diseases that impact 1 in 2,000 individuals, collectively affecting an estimated 400 million people globally. Thirty percent of children diagnosed with rare diseases do not survive beyond the age of five, while 35% face the challenges of reaching their first year of life. The lack of a known cure for 90% of these diseases leads to substantial psychosocial and economic burdens for patients, their families, and healthcare systems. It is known that 80% of rare diseases have a genetic basis, suggesting that the parents of an affected child carry the disease-causing mutation [1]. Unfortunately, couples often only realize that they are carriers of a genetic disease when they have an affected child. In contemporary medicine, preventive treatment methods ensure the identification of risks before diseases occur. In this context, screening for rare diseases commonly observed in individuals before marriage and identifying

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couples carrying genetic diseases are of great importance in the scope of public health services, aiming to provide healthy reproductive options.

Carrier testing first introduced in the 1970s, aiming to screen prospective parents for autosomal and X-linked diseases. The initial carrier screening programs were applied exclusively to ethnic groups, screening for Tay-Sachs disease in Ashkenazi Jewish communities and Beta Thalassemia in Mediterranean populations. In 1993, the inclusion of pan-ethnic Cystic Fibrosis in carrier screening programs, regardless of family history and ethnic background, brought the term "Universal Carrier Testing" into consideration [2]. In 2010, Consyl, a biotechnology company, introduced a screening test encompassing 100 Mendelian inherited genes. Subsequently, in 2011, Bell et al. achieved a milestone by being the first group to employ Next-Generation Sequencing (NGS) technology in a screening panel comprising 437 genes [3].

Research and discussions are ongoing to come up with universal carrier screening tests; which have become a widely used approach for detecting couples at increased risk of having an affected child due to ethnicity or consanguineous marriage; in terms of scope and effectiveness. The initial approach of hot spot screening for common mutations has been replaced by NGS-based extended screening tests that cover 100-400 diseases. After recent developments in genomic technologies, condition-specific carrier screening has been replaced Expanded Carrier Screening (ECS) approach, which enables screening for a large number of genetic diseases independent of ethnic background. Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS), which are now being incorporated into screening programs, aim to screen for all diseases, including rare conditions in genetically isolated populations [4]. In genetic laboratories, instead of generating a comprehensive report for all diseases, the common approach is to generate a virtual panel for a specific set of genes and examine exome data for mutation analysis [5]. Developments in NGS technology have led to a significant increase in genetic data. The increase in genetic testing options, especially including WES and ECS panels, has led to a complex growth in the number variants that are reported. This data growth has led to a need for a standardization in variant reporting.

Correct analysis and interpretation of these data and reporting of variants associated with the patient's phenotype are critical for making the correct diagnosis. Test results for molecular genetic diagnosis of single gene disorders are based on the classification of variants according to their disease-causing effects. This classification is made in line with the criteria recommended by the "American College of Medical Genetics (ACMG)" [6,7]. Variants are classified as pathogenic (Class 1), likely pathogenic (Class 2), “Variant of Unsignificance” (VUS or Class 3), likely benign and benign based on these criteria. Nonetheless, the interpretation of “Variant of Unsignificance” (VUS), which represents the most challenging outcome of the extensive variant data derived from NGS technologies, adds complexity to the test results. Therefore, only pathogenic (Class 1) and likely pathogenic (Class 2) variants are filtered and reported in exome and genome-based tests [6,7]. Cost-effectiveness and a single workflow for all genetic testing refer to the efficiency and economic effectiveness of using WES and WGS methods as universal tests for various genetic disorders. In this context, to improve cost-effectiveness and ensure an efficient turnaround time, reference laboratories are obligated to process multiple samples sequentially on high-throughput NGS devices. For this purpose, genetic laboratories aim to optimize genome-based carrier screening tests to increase the number of samples processed. However, current clinical applications of NGS lack the sensitivity and specificity required for detecting all types of variants, such as those leading to triplet repeat disorders (e.g., Fragile X), and specific genome regions remain challenging (pseudogenes) for current clinical NGS applications.

The current short-read approach in widely used NGS platforms presents limitations in clinical laboratories, requiring multiple methods to detect the full range of variant classes. However, the anticipated introduction of third generation long-read DNA sequencing technologies, along with advances in bioinformatics pipelines for detecting short tandem repeats and copy number variations will soon overcome these challenges in the clinical laboratory. Genomic sequencing surpasses the constraints associated with the capture or amplicon-based sequencing methodology. At the same time, it avoids the drawbacks of PCR-based library preparation, which can be a source of variant artifacts and PCR bias, resulting in a non-uniform representation of the DNA library. Genome sequencing has the additional advantage of accurately identifying the exact breakpoint in the DNA sequence, facilitating the detection of structural variations in the genome [8].

While genomic sequencing technology is advancing rapidly, the pace of data interpretation needs to catch up. The complexity arises from the interpretation of variants needing to be reported clinical relevance, representing the most challenging aspect of handling big data, and thus complicates the results of these tests.

Carrier Screening Strategies

Ethnic-specific, pan-ethnic, and ECS are different preconception and prenatal carrier screening strategies. Ethnic-specific carrier screening or ethnic-based screening aims to screen a specific high-risk ethnic group for increased risk of disorders, for example, screening those of the Ashkenazi Jewish population for Tay–Sachs disease [9]. The pan-ethnic (nondirective) approach screens for a panel of disorders in all individuals, regardless of ethnic origin. ECS is not well defined, and sometimes its definition is confusing. Some carrier screening test strategies use the term "expanded" to screen a large number of genes (>100 genes), some are used to screen populations with higher carrier frequencies, and the other ECS strategy screens many more variants within a gene. Since different panel definitions may create some difficulties in the genetic counseling of patients about carrier screening tests, ACMG recommends a classification system for carrier screening tests. ACMG recommends that it is more appropriate to use the term carrier screening instead of ECS. ACMG classified carrier screening tests into four tiers. Tier 1 defines the screening for Cystic Fibrosis (CF) and Spinal Muscular Atrophy (SMA) regardless of ethnicity or population [10,11], Tier 2 tests screen the conditions with ≥1/100 carrier frequency (includes Tier 1). Tier 3 tests screen the conditions with ≥1/200 carrier frequency (includes Tier 2) and X-linked inheritance. Tier 4 tests aim to screen the conditions with <1/200 carrier frequency (includes Tier 3), and genes/conditions might vary between laboratories.

The ACMG recommends that all patients who are pregnant or planning to become pregnant should be offered Tier 3 carrier screening tests. Patients with a family or personal history and pregnancies of known or potential consanguineous marriages should be offered Tier 4 screening. ACMG does not recommend the use of Tier 1 and/or Tier 2 because they do not provide evaluation of all racial/ethnic groups. ACMG does not recommend the use of Tier 1 and/or Tier 2 because they do not provide evaluation of all racial/ethnic groups. The Tier 4 test should not be used for routine analysis and should only be offered for defined populations.

Which conditions should be screened?

Carrier screening programs should define the selection criteria for screening conditions [9,12,13]. Two leading organizations, ACMG and ACOG, regularly publish guidelines on carrier screening. ACMG offers several criteria for determining the content of carrier test panels. They defined a gene list containing 86 genes related to autosomal recessive diseases [10]. The gene lists were defined according to the

carrier frequency of the diseases. The gene list consist of genes with a carrier frequency ≥1/50 (19 genes), genes with a carrier frequency <1/50 to ≥1/100 (19 genes), genes with a carrier frequency<1/100 to ≥1/150 (25 genes), genes with a carrier frequency <1/150 to ≥1/200 (23 genes) and genes that were offered for screening outside of the gnomAD criteria (11 genes). Criteria for gene selection were derived from gnomAD. Carrier frequency of at least 1/200 for six ancestral populations and genes with at least a 1/200 carrier frequency of pathogenic or likely pathogenic variants in a subpopulation with at least 1% representation in the US, including US territories, were selected. The X-linked conditions (16 genes) were selected based on prevalence data from OMIM, Orphanet, or MedlinePlus. The ACMG recommends that individuals considering pregnancy or who are already pregnant undergo level 3 carrier screening, which includes at least 86 autosomal recessive conditions and 16 X-linked conditions. ACOG recommends screening for conditions with a carrier frequency of ≥ 1/100 [12,13].

The selection of genes is determined by the clinical severity of the conditions that affect the reproductive decision. The ACMG recommends that diseases of profound, severe or moderate severity be included in carrier screening panels [9]. The clinical severity of the disease is defined as follows; profound: shortened life span in infancy or childhood, intellectual disability; severe: lethal in early adulthood, impaired mobility, or malformation of an internal organ; moderate: neurosensory impairment, immune deficiency or cancer, intellectual disability, dysmorphic features [9,14,15]. According to ACOG, the diseases that should be included in the panels are as follows: those that have a detrimental effect on the quality of life, diseases that cause cognitive or physical impairment, and diseases that require surgical or medical intervention and begin early in life [12,13].

Carrier screening panels should include conditions with a strong gene-disease association [9,14,15]. The conditions with supportive evidence of genotype-phenotype association are appropriate for carrier screening panels, but the conditions with limited gene-disease association should not be included. Guidelines recommend that panels should screen for prenatally diagnosed conditions or the conditions that can be screened by Preimplantation Genetic Testing (PGT) [9,12-15].

Counselling/Reporting

Patient education and genetic counseling are crucial components of carrier screening tests [9,14-16]. Health care professionals should provide comprehensive genetic counseling both before and after testing, discussing the risks, benefits, and consequences of the screening process. Additionally, couples should be informed about the testing method, limitations, and potential consequences [9,14,15]. Informed consent is essential and should be obtained from all individuals tested. Informed consent should include information about the carrier's reproductive risks for AR and XL conditions. Individuals should be counseled that the carrier status of a person with a positive result does not usually affect their own health. They should also be informed about the potential of the test to identify incidental variants that may cause health risks or lead to possible diagnoses [9,12,14,15]. The informed consent should clearly state the criteria for reporting VUS, and only those variants that meet these criteria should be included in the report. Patients should be aware that variant classifications may evolve over time as databases are updated. Genetic counseling should include encompass conversation of reproductive options such as prenatal diagnostic procedures or PGT [14,15]. Fetal diagnosis should be recommended if the test is positive during pregnancy.

Carrier screening cannot detect all hereditary conditions and as a result, it cannot completely rule out the possibility of being a carrier for such conditions. It is crucial to communicate to couples that even with a negative result, the risk of being a carrier can never be reduced to zero. Negative test result might be due to following reasons [9,15];

- Not all genes contributing to a particular disease may be identified
- The screening panel may not encompass all genes associated with the disease.
- The method employed may not cover all regions of a gene
- The screening technology may lack the capacity to detect all causative variants.
- There is a possibility of incorrect classifications of variants.

Results

Next Generation Sequencing (NGS) has become the state-of-the-art technology for carrier screening with low turnaround time and high throughput. Pan-ethnic testing has only been recommended for Hemoglobinopathies, Cystic Fibrosis, and Spinal Muscular Atrophy. Whole genome/exome sequencing is also at reach but due to the size of the sequenced region, considering multiplexing requirements to maintain cost-effectiveness, their sensitivity for challenging regions may fall and may require additional tests for ensuring analytical validity.

In carrier screening, the focus has been on specific conditions that are more prevalent in certain ethnic groups. The significance of ECS becomes evident when we consider that five new genetic diseases are described in the scientific literature every week. The growing prevalence of multi-ethnic couples in the United States and the rising diversity of populations in Europe, driven by increased waves of immigration, highlight the demand for ECS tests. Still, there is no consensus on the "ideal" ECS design. The optimization of disease risk sensitivity and specificity differs from a panel-design strategy that maximizes the number of genes. A recent idea is that specificity, sensitivity, and uniform coverage are more important than the number of genes involved in the panel.

Discussion

In the investigation involving 100 consanguineous couples, a virtual panel consisting of 2138 genes was generated, and exome-based expanded ECS was performed. Thirty novel carrier conditions, not previously identified in the couples or their offspring, were observed in 28 out of 100 couples, resulting in a novel finding diagnostic rate of 28%. In the study group, total of 58 out of 100 consanguineous couples are carriers of at least one autosomal recessive disease, and among them, 19 couples had previously undergone PGT [15]. The findings of this study underscore the significant influence of utilizing comprehensive gene panels on outcomes, particularly in consanguineous marriages. With this approach, the number of cases suitable for PGT can be increased, thus contributing to the eradication of rare diseases by facilitating diagnosis for more couples.

The pre-marital screening programs aim to protect families and society from the psychological and socio-economic consequences of having an affected child, and future generations from premature deaths, physical suffering, psychological burden, and years of undiagnosed and untreated life. Especially in communities with a high rate of consanguineous marriages, the risk of giving birth to children with rare diseases increases. Along with the differentiation of the genetic pool of populations due to migration waves, new genetic diseases can also emerge. In the search for the ideal carrier screening test, we have high expectations for specificity, sensitivity, and uniform coverage of target genes. This includes screening for a wide range of genetic diseases and the ability to assess single nucleotide variants (SNVs), insertions and deletions (indels), and copy number variations (CNVs). In addition, the ideal test should be able to detect novel variants across the en-

tire region of interest, including hotspots in intergenic and intronic regions. Classification of all detected variants is another critical aspect [17].

In contrast, the contemporary perspective suggests that specificity, sensitivity, and uniform coverage are more important than the total number of genes included in the panel. The current strategy suggests that a test that covers 100 diseases with 100% sensitivity is more effective than a test that covers 1000 diseases with only 10% sensitivity. This paradigm shift underscores the importance of precision and comprehensive coverage in achieving optimal screening results [16]. Targeted Expanded Carrier Screening (T-ECS) tests offer significant advantages over exome and genome-based ECS panels. These tests are cost-effective, focused, and provide rapid results. Interpretation of T-ECS results is generally straightforward due to the limited number of genes analyzed. However, a significant drawback is the potential exclusion of novel or rare variants outside the selected panel, requiring frequent updates and revisions.

Conclusion

Because exome and genome-based ECS tests have comprehensive coverage, they can identify a wider range of variants. It is well known that the more genomic data is obtained, the more challenging its interpretation becomes. Each approach has its merits and limitations, and the choice should be tailored to individual patient needs and circumstances.

The American Society of Medical Genetics and Genomics (ACMG) recommended reporting secondary findings for these 78 genes in the new guideline published by ACMG in 2021 regarding late-onset diseases (cancer, cardiovascular and neurodegenerative diseases) in 2016 [9,17]. In addition to these guidelines, these genes have recently begun to be included in expanded carrier panels. These advances in the field of genomics have led to the detection of many new variants related to late-onset conditions and hereditary cancers.

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