

# Clinical experience with multigene carrier panels in the reproductive setting

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## Abstract

**Objectives:** Expanded carrier testing is acknowledged as an acceptable strategy for carrier testing by the American College of Obstetrics and Gynecology. Limited studies have investigated positivity rates of expanded carrier panels. We describe our experience with 3 commercial laboratory panels varying in size from 3 to 218 disorders.

**Methods:** We reviewed outcomes for 3 multigene carrier screening panels: trio (3 diseases), standard (23 diseases), and global (218 diseases). All panels used targeted genotype analysis of preselected mutations via next-generation sequencing. We calculated positivity rates for each panel.

**Results:** Positivity rates were 7.2% for Preparent Trio, 13.2% for Preparent Standard, and 35.8% for Preparent Global. The most frequent positive results in the global panel were (in descending order): abnormal hemoglobin electrophoresis, familial Mediterranean fever, cystic fibrosis, fragile X, glucose-6-phosphate dehydrogenase deficiency, alpha-thalassemia, and nonsyndromic hearing loss.

**Conclusions:** While genetic diseases are individually rare, they are cumulatively common. Our experience illustrates that, with a panel of 218 diseases, the likelihood of identifying a carrier can be as high as 36%. Understanding panel positivity rates is one important factor for providers when choosing the right test for their practice, setting appropriate expectations for patients, and planning for follow-up counseling.

## 1 | INTRODUCTION

Autosomal recessive and X-linked genetic variants contribute to human diseases. The purpose of carrier testing is to identify couples who are at risk for having a child with a genetic disease. On average, a person will carry 2 to 3 pathogenic genetic variants that can lead to known lethal recessive conditions in offspring.<sup>1</sup> Once an increased risk is identified for a couple or individual, they can be offered preconception reproductive planning or prenatal testing options as well as counseling and

information regarding future medical management for an affected child. Carrier couples identified before conception have multiple reproductive options including preimplantation genetic diagnosis with in vitro fertilization, donor gametes, adoption, prenatal diagnostic testing, or the choice of no testing or intervention.<sup>2</sup> For these reasons, discussing carrier testing options prior to pregnancy is ideal but still an important part of prenatal care after a person has become pregnant.

Historically, it was recognized that certain genetic conditions were more prevalent in specific ethnicities. For example; hemoglobinopathies are more common in the African, Mediterranean, and Southeast Asian populations, and Tay-Sachs disease is more prevalent in the Ashkenazi

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Jewish population compared with the general population.<sup>3,4</sup> Cystic fibrosis (CF), spinal muscular atrophy (SMA), and a CBC with red blood cell indices to screen for hemoglobinopathies and thalassemias are the only population-based carrier testing recommendation due to disease prevalence and severity.<sup>5-7</sup> Carrier testing for all other genetic conditions relied on patient inquiry or a positive family history and/or known familial mutation(s) to prompt a provider to order testing. Recently updated guidelines acknowledge that ethnic-specific, pan-ethnic, and expanded panels are all acceptable carrier testing strategies taking into account changing patterns of ethnicity and innovations in genetic testing.<sup>2</sup>

Advances in genomic technology allow for efficient simultaneous sequencing of many genes and expanded carrier testing is now deemed an acceptable strategy by American Congress of Obstetricians and Gynecologists (ACOG).<sup>7</sup> Recent commentary from professional societies has addressed the potential benefits of this testing methodology while also highlighting potential considerations for implementation.<sup>8,9</sup> Benefits of expanded carrier testing include less of a need for complete or accurate knowledge of one's ancestry in an increasingly multiethnic society; pan-ethnic screening for genetic conditions that do not occur solely in specific ethnicities; and testing for multiple conditions at one time, which increases the amount of accessible genetic information for screening participants.<sup>8,9</sup>

Additionally, expanded carrier testing will identify more at-risk couples and further reduce diagnostic odysseys for affected children compared with traditional carrier testing. Currently, providers and their patients have several options to choose among for carrier testing. We present our clinical experience with 3 multigene carrier testing panels performed at Progenity, Inc, ranging in size from 3 to 218 genes. We explore the positivity rates of these 3 panels as well as the most commonly identified disorders on the largest panel. The goals of this study were to identify the positivity rates for each panel and the disorders most commonly found to be positive in the population tested. This information will allow for improved reproductive counseling, as it pertains to carrier testing.

## 2 | METHODS

We reviewed outcomes for 3 multigene carrier testing panels. This was a retrospective analysis without patient identifiers; therefore, ethical approval was waived. All panels used targeted genotype analysis of preselected mutations using a laboratory-developed test that uses 5'-phosphorylated single-stranded DNA capture probes to prepare targeted libraries for massive parallel sequencing.

Briefly, DNA is extracted from blood, mouthwash, or buccal samples and added to 96 well microtiter plates containing a mixture of the targeted DNA capture probes. Polymerase and ligase are added, and an extension and ligation reaction is performed creating single-stranded DNA circles. Extension and ligation is followed by exonuclease digestion to remove excess probe and genomic DNA. Next, a unique molecular barcode is attached to each sample along with Illumina sequencing adaptors (Illumina, San Diego, California). To determine DNA concentration, a second plate containing a portion of the barcoded DNA is pooled; purified via AmPureXP (Beckman Coulter, Brea, California); and quantified using the Qubit dsDNA High

### What's already known about this topic?

- Expanded carrier testing is available as an assessment option.
- Pan-ethnic-based testing does not rely on ethnicity as a qualification for assessing the presence of specific genetic conditions.

### What does this study add?

- This is a report of positivity frequencies for three 3 multigene carrier panels offered by a single laboratory ranging from 7.2% to 35.8%.
- The study includes a list of the most commonly positive conditions on a large expanded carrier panel.

Sensitivity Assay (Thermo Fisher, Waltham, Massachusetts). Once the pooled library passes quality control, the pooled library is diluted, denatured, and loaded onto an Illumina HiSeq 2500 Rapid v2 flow cell under the HiSeq Rapid Run mode. The Illumina HiSeq 2500 is run using paired end, 106 base pair chemistry. Data are aligned and analyzed via a custom progenity sequence analysis algorithm.

Fragile X trinucleotide repeats were assessed using the Asuragen AmplideXTM FMR1 PCR Kit (Asuragen, Austin, Texas), which is based on a 3-primer CGG repeat primed polymerase chain reaction (PCR) from purified genomic DNA followed by fragment sizing on the Applied Biosystems 3500 XL DX capillary electrophoresis platform (Applied Biosystems, Foster City, California). Spinal muscular atrophy carrier status is assessed via SMN1 dosage analysis. The standard and global panels also included hemoglobinopathy evaluation (mean corpuscular volume (MCV) and hemoglobin electrophoresis) and hexosaminidase-A enzyme analysis for Tay-Sachs disease (Quest Diagnostics, Wood Dale, Illinois, and Baylor College of Medicine, Houston, Texas, respectively).

The Preparent Trio panel assesses for cystic fibrosis, spinal muscular atrophy, and fragile X. The Preparent Standard panel tests for 23 conditions, and the Preparent Global panel tests for 218 conditions, which are outlined in the supplemental materials. Disorder lists for these panels can be found in the Supporting Information section.

A result was considered positive if it fit into at least one of the following categories: mutation detected; hemoglobin electrophoresis suggestive of hemoglobinopathy carrier or abnormal red blood cell indices suggestive of thalassemia carrier (reduced MCV, reduced mean corpuscular hemoglobin (MCH), reduced hemoglobin); hexosaminidase A enzyme level suggestive of Tay-Sachs carrier; fragile X premutation carrier; fragile X full mutation; and/or fragile X gray zone carrier. All other results were considered negative.

All results were analyzed and designated as a negative or positive result based on previously described criteria. Positivity rates for each panel were calculated. The positive results received from the global panel were queried by disease to determine the most frequent

**TABLE 1** Participant reported ethnicity demographics for the three panels

Ethnicity	Trio	Standard	Global
African-American/black	9.9%	16.7%	5.9%
Ashkenazi Jewish	0.2%	0.5%	3.1%
Asian	5.6%	3.9%	10.7%
Caucasian/white	49.7%	32.7%	47.0%
Hispanic	18.9%	33.8%	9.1%
N/A	0.7%	0.5%	0.6%
Native American	0.1%	0.1%	0.1%
Other or mixed	14.8%	11.7%	23.2%
Sephardic Jewish	0.0%	0.0%	0.3%
Total	100%	100.0%	100.0%

positive conditions on that panel; positivity rates for each individual condition were also calculated.

### 3 | RESULTS

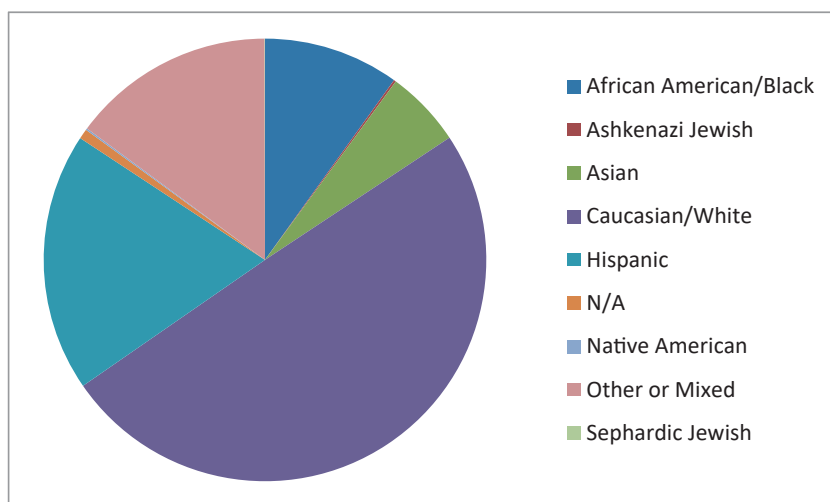
Data were available from a total of 75 036 assessed participants. All participants were referred for testing by a provider in the United States. Indications for testing were not evaluated.

#### 3.1 | Trio panel

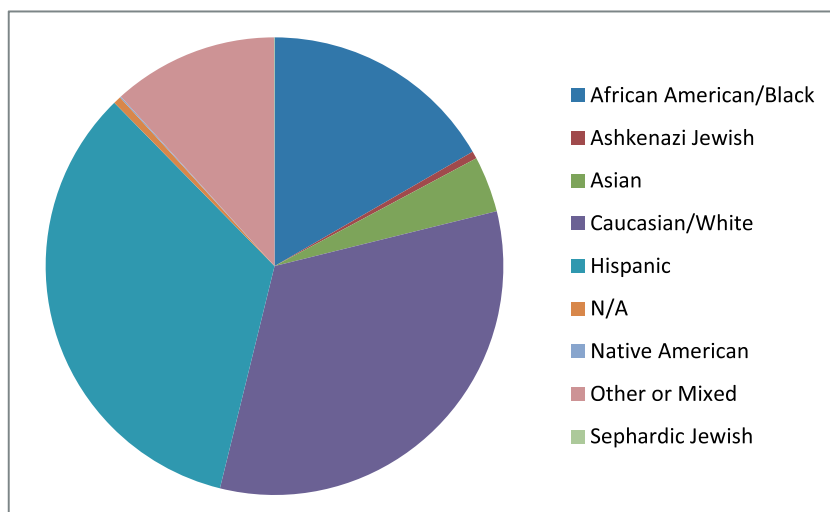
A total of 51 584 samples were tested via the trio panel; A total of 51 117 participants were female, 406 were male, and 1 participant did not select a sex. The breakdown of ethnicities can be found in Table 1 and Figure 1. Nearly half of the participants identified with Caucasian/white ethnicity. A total of 3707 patients had a positive result, which equates to a 7.2% positivity rate.

#### 3.2 | Standard panel

A total of 19 550 samples were tested via the standard panel; 19 082 participants were female, and 468 were male. The most common reported ethnicity of the participants who were tested via this panel was Hispanic (Table 1 and Figure 2). We queried the percentage of each panel ordered by region of the country (Table 2). Of note, 45.7% of orders originated from the Texas region, which is composed of Texas and Oklahoma, were standard panels. Overall, 2576 patients tested via the standard panel were positive for a known deleterious mutation, fragile X gray zone, hexosaminidase A enzyme analysis suggestive of Tay-Sachs carrier status, and/or abnormal hemoglobin electrophoresis. The standard panel yielded a 13.2% positivity rate.



**FIGURE 1** Trio panel participant demographics. Caucasian/white composed most participants at 49.7%. Hispanic (18.9%), other/mixed (14.8%), African-American/black (9.9%), and Asian (5.6%) accounted for 49.2% in total



**FIGURE 2** Standard panel participant demographics. Hispanic was the most selected ethnicity comprising of 33.8% of participants; 32.7% of the participants identified as Caucasian/white

**TABLE 2** Frequency of panels ordered by region<sup>a</sup>

Region	Trio %	Standard %	Global%	Total
Great Lakes	68.3%	30.4%	1.3%	100.0%
Mid Atlantic	66.7%	18.3%	15.0%	100.0%
Midwest	87.0%	5.2%	7.9%	100.0%
Mountain plains	76.6%	18.5%	4.9%	100.0%
NorthEast	89.2%	7.4%	3.4%	100.0%
SouthEast	85.4%	12.0%	2.6%	100.0%
Texas	53.1%	45.7%	1.2%	100.0%
West	62.2%	18.1%	19.8%	100.0%
Total:	69.0%	25.8%	5.2%	100.0%

<sup>a</sup>For example, 68.3% of panels ordered out of the Great Lakes region were trios, 30.4% were standard, 1.3% were global.

### 3.3 | Global panel

A total of 3902 samples were tested via the global panel; 3279 were female, and 623 were male. The breakdown of participant ethnicity can be found in Figure 3 and Table 1. The most commonly selected ethnicity was Caucasian/white followed by other/mixed. A total of 1395 patients tested positive for at least 1 condition, resulting in a 35.8% positivity rate.

### 3.4 | Most common conditions

The most commonly positive conditions on the global panel in descending order were positive hemoglobin electrophoresis (5.5% positivity), familial Mediterranean fever (5.2%), cystic fibrosis (3.3%), fragile X (2.6%), glucose-6-phosphate dehydrogenase deficiency (2.4%), alpha-thalassemia (2.1%), GJB2-related nonsyndromic hearing loss (1.8%), primary congenital glaucoma (1.7%), spinal muscular atrophy (1.6%), medium-chain acyl-CoA dehydrogenase deficiency (1.4%), abnormal hexoasaminidase A enzyme suggestive of Tay Sachs carrier (1.1%), and congenital disorder of glycosylation type IA (1.0%) (Table 3). In total, 127 conditions came up positive at least once in this cohort.

**TABLE 3** The most commonly positive conditions on the global panel and the positivity rate for each condition

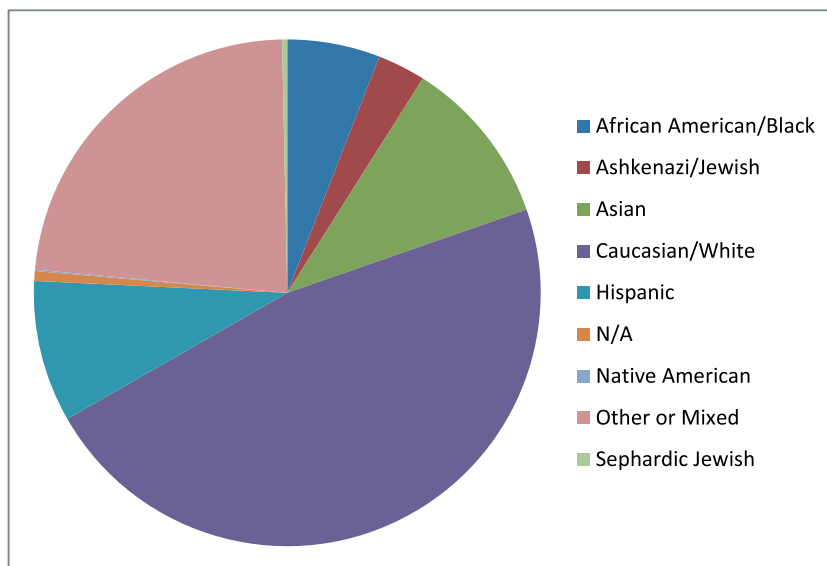
Disease	Positivity
Hemoglobin electrophoresis	5.5%
Familial Mediterranean fever	5.2%
Cystic fibrosis	3.3%
Fragile X	2.6%
Glucose-6-phosphate dehydrogenase deficiency	2.4%
Alpha-thalassemia	2.1%
Nonsyndromic hearing loss, GJB2-related	1.8%
Primary congenital glaucoma	1.7%
Spinal muscular atrophy	1.6%
Medium-chain acyl-CoA dehydrogenase deficiency	1.4%
Tay Sachs disease -Hexosaminidase A	1.1%
Congenital disorder of glycosylation, type IA	1.0%

### 3.5 | Demographic observations

Differences in ordering patterns were observed between ethnic groups and between geographic regions in the United States (Tables 1 and 2). Participant-reported ethnicity demographics demonstrated some interesting differences among the panels. The frequency of Hispanic and African-American patients were increased for the standard panel compared with the trio and global panels.

We also observed that the percentage of patients identified as having "Other or Mixed" ethnicity was highest in the global panel. Previous studies have demonstrated 2 relevant points: Mixed ethnicity is increasingly more common in the US population, and patients are also increasingly unable to correctly identify their own ethnicity or ethnicities.<sup>10,11</sup> Thus, offering carrier testing based solely on a person's reported ethnicity has inherent limitations. While certain disorders are more frequent in people of specific ethnic backgrounds, those disorders do not occur only in those populations.

Differences in regional ordering patterns were observed. Trio panel was the most commonly ordered panel in all regions of the country. Notably, in the Texas region, 45.7% of panels ordered were standard

**FIGURE 3** Global panel participant ethnicity demographics. Caucasian/white was the most selected ethnicity comprising of 47% of participants. Other/mixed was the second most common ethnicity totaling 23.2%

panels, which was higher than the national average of 25.8%. A trend towards ordering larger panels was observed in the Mid-Atlantic and West regions where the percentage of global panels were 15.0% and 19.8%, respectively, compared with the national average of 5.2%.

## 4 | DISCUSSION

Genetic diseases are individually rare, but common when considered cumulatively, and carrier testing positivity rates, as demonstrated by this study and others, reinforce this fact. Our study demonstrated that the trio, standard, and global panels yielded 7.2%, 13.2%, and 35.8% positivity, respectively. In other words, when testing with a 200+ disease panel, as many as 1 in 3 patients screened will have a genetic carrier risk identified.

It is not surprising that larger panels are associated with higher positivity rates, but there are both benefits and challenges for patients and clinicians receiving this information. Our global panel provides more genomic information to patients, which can be desirable for women and couples who want a great deal of information available for reproductive decision-making. Larger panels may also be desirable for providers who want to uncover genetic risk or provide reproductive risk reduction on a large scale for their patients.

However, larger panels with higher positivity rates require increased clinical support. Many of the diseases on carrier testing panels have autosomal recessive inheritance. This means that, when parents are screened sequentially, a positive carrier result in one parent triggers carrier testing in the other parent. A clinical workflow must be supported so that testing results are communicated and follow-up partner testing is coordinated. Panels that include X-linked recessive disorders may also identify carriers that manifest some phenotypic expression of the disease. The intent of carrier testing is to provide couples with information about reproductive risk and options, but informed consent procedures should include information relating to other possible outcomes of testing such as minor phenotypic expression.<sup>2</sup> Educational resources for both patients and providers are also needed, as larger carrier panels will identify carrier status for both common and rare diseases. While common diseases such as cystic fibrosis and hemoglobinopathies are familiar to prenatal providers, many rare diseases are not, and educational support may be needed to ensure that accurate clinical information is available to the provider and communicated to the patient so they can make informed decisions. Finally, identification of local resources is also important to build a referral network that supports related clinical care. This network may include, but is not limited to, genetic counselors, reproductive endocrinologists, maternal fetal medicine specialists, and the laboratories performing the carrier testing and related genetic testing. For all of these reasons, carrier testing panel positivity rates are an important consideration when practices select a preferred panel and create a supportive clinical workflow. Laboratories offering carrier testing panels should help set appropriate expectations about panel positivity rates.

### 4.1 | Demographic observations

The frequency of Hispanic and African-American patients were increased for the standard panel compared with the other panels. The standard panel was purposefully developed to include all

conditions for which there are specific carrier screening guidelines: CF, SMA, fragile X, hemoglobinopathies, and Ashkenazi Jewish diseases. Several factors may be contributing to the relative overrepresentation of Hispanics and African-Americans in this group. Hemoglobinopathy carrier testing is recommended by ACOG for these ethnicities. Therefore, the inclusion of that analysis may increase the likelihood that a provider would choose this panel over a trio. It is also possible that a disproportionate number of samples in this cohort were received from Texas. According to the 2015 census, the population of Texas is 38.8% Hispanic.<sup>12</sup> This overrepresentation likely contributes to the skewed experience of our laboratory.

Other or mixed ethnicity was highest in the global panel. The trend toward larger panels in patients of other or mixed ethnicity may be predictive of the pan-ethnic approach that will likely become standard of care as population admixture continues to increase.

Differences in regional ordering patterns were observed (Table 2). These trends may be due to several factors including sales practices; regional patient preferences and trends; and regional provider ordering preferences. Genetic carrier testing is optional and always an individual patient choice. However, clinic and provider preferences and protocols may vary by region. For example, in certain clinics in California, the protocol may be to offer all patients a global panel, whereas in middle America, the protocol may be to offer trio to all patients and standard and global as indicated by patient ethnicity and family history. Thus, regional ordering trends likely reflect regional provider protocols.

### 4.2 | Limitations

This is a retrospective study and comprises the experience of 1 commercial laboratory. Indications for testing were not evaluated, and some patients may have been tested because of a family history, which would result in a higher likelihood of a positive result. Business factors, such as increased sales in certain geographic regions, may shift the demographic characteristics of the tested population and skew the view of ordering patterns. This laboratory experience was limited to samples tested within the United States, and there may be a limited ability to generalize our findings to other countries. Positivity rates are impacted by detection rates, and like all carrier testing panels, detection rates are not uniform across all diseases tested. The assay used for this study is not diagnostic, and follow-up information was not collected. Specifically, some positive hemoglobinopathy evaluations may have been due to iron deficiency anemia and not alpha-thalassemia trait status. Therefore, we cannot say for certain that all patients with positive hemoglobinopathy results were true carriers; however, they were treated as at risk since follow-up testing to further define their carrier status was recommended. This supports our conclusion that increased clinical support and education should be a consideration when choosing panel testing.

### 4.3 | Future directions

While population-based carrier testing for some diseases has been a routine part of prenatal care since the 1970's, the increasing use of

expanded carrier testing panels and ACOG's latest carrier testing committee opinion offers many opportunities for further research.<sup>4</sup> Large data sets of carrier testing in the general population offer an opportunity to compare expected versus observed carrier frequencies for both common and rare diseases. As carrier testing protocols evolve, there will also be opportunities to better understand the benefits and challenges of expanded carrier testing for both clinicians and patients and the effect on overall medical care cost. These insights will allow the clinical community to define and implement best practices that ensure good patient outcomes and efficient clinical workflows. Additionally, demonstrating the number of dual carrier couples (a couple who are carriers of the same disorder) identified will highlight reproductive implications of expanded carrier screening. Thus far, this aspect of analysis has been theoretical.<sup>13</sup>

## 5 | CONCLUSION

This study explored the experience of a commercial laboratory offering carrier testing panels of varying sizes to clinical populations in the United States. This analysis demonstrated that positivity rates vary significantly by panel size, with up to 1 in 3 patients testing positive on the largest (200+ disease) panel. Expanded carrier testing panels are an effective way to identify carriers of genetic diseases, particularly in ethnically diverse populations. Laboratories offering carrier testing should communicate their positivity rates and provide support for clinician and patient education, as appropriate.

## CONFLICT OF INTEREST

C.T., N.T., R.A., L.D., C.S., and C.H. are full-time employees of Progenity, Inc. R.L. has no conflicts.

## FUNDING SOURCES

None.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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