# Carrier Screening with Next-Generation Sequencing Detects Common, Uncommon, and Novel Mutations

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## Background

Carrier screening for specific genetic disorders is recommended by the American Congress of Obstetricians and Gynecologists (ACOG), the American College of Medical Genetics and Genomics (ACMG), and the American Society for Reproductive Medicine (ASRM). Traditional carrier screening assays are designed to look for only the most common mutations within a gene, due to cost considerations and restrictive technologies. While this can yield high detection rates in specific populations (e.g., the Ashkenazi Jewish), the detection rates outside of these ethnicities or in patients of mixed ethnic background are often suboptimal. As carrier screening becomes a more routine practice, and ethnic background is not always clear to define, it is becoming exceedingly important to develop carrier screening assays that are accurate, regardless of a patient's ethnic background.

Next-generation DNA sequencing (NGS) is a technology that is able to detect more mutations than traditional carrier screening assays, therefore it should provide a more comprehensive determination of carrier status, regardless of a patient's ethnic background.

## Objective

Our objective was to evaluate the clinical effectiveness of NGS in screening for carriers of 14 society-recommended disorders across a large number of patients in a clinical setting.

## Design

Using NGS, we evaluated carrier status for up to 14 diseases (as ordered by physicians) for 71,070 patients from IVF centers across the US.

Diseases screened, and corresponding genes in parentheses, may include: Bloom's syndrome (*BLM*), Canavan disease (*ASPA*), cystic fibrosis (*CFTR*), dihydrolipoamide dehydrogenase deficiency (*DLD*), familial dysautonomia (*IKBKAP*), familial hyperinsulinism (*ABCC8*), Fanconi anemia group C (*FANCC*), glycogen storage disease 1a (*G6PC*), maple syrup urine disease type 1A/1B (*BCKDHA/B*), mucolipidosis type IV (*MCOLN1*), Niemann-Pick type A/B (*SMPD1*), Tay-Sachs disease (*HEXA*), Usher syndrome 1F (*PCDH15*), Usher syndrome 3 (*CLRN1*).

**Table 1**: Distribution of ethnicity in study population of 71,070 patients

| Self-reported Ethnicity | % of Total |
|---|---|
| Caucasian | 39.2 |
| Not Provided | 34.7 |
| African American | 6.7 |
| Asian | 6.2 |
| Hispanic | 5.2 |
| Mixed | 3.3 |
| Other* | 3.0 |
| Ashkenazi Jewish | 1.6 |

*Mediterranean, Sephardic, French Canadian

## Results

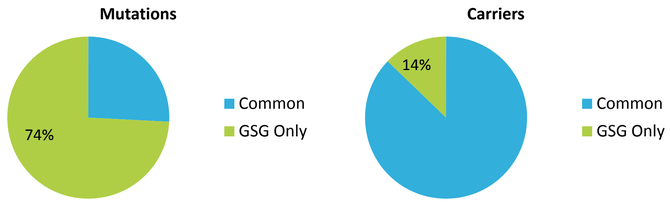
Our NGS platform detected a total of 3,093 mutations (403 distinct mutations) among 15 genes responsible for 14 diseases, including numerous mutations missed by other screening assays. Had traditional screening assays been used, 13.9% (95% CI: 12.7–15.2) of all carriers identified across these diseases would have been missed. Excluding cystic fibrosis (a well-characterized disease), 30.6% (95% CI: 27.8–33.5) of carriers would have been missed, putting the reproductive couple at increased risk of having a child with a genetic disorder.

## Materials and Methods

Carrier status was evaluated using a proprietary and validated NGS methodology (comprised of multiplex gene capture, sequencing, and sophisticated computational analysis) which determined the presence or absence of pathogenic mutations. Pathogenic mutations include common and rare previously-reported disease-causing changes, as well as novel (previously-unreported) truncating changes.

**Figure 1** and **Table 2:** Percentage of different carriers and mutations detected by different screening approaches. We define common mutations as those detected by traditional carrier screening assays. GSG Only includes both rare previously-reported mutations and novel mutations.

Figure 1




**74% of distinct mutations would be missed using genotyping-based carrier screening**

Table 2

| Gene | Common[1] | | GSG Only | | Total | |
|---|---|---|---|---|---|---|
| | Mutations | Carriers | Mutations | Carriers | Mutations | Carriers |
| *ABCC8* | 3 | 41 | 17 | 21 | 20 | 62 |
| *ASPA* | 3 | 73 | 12 | 14 | 15 | 87 |
| *BCKDHA/B* | 3 | 50 | 33 | 51 | 36 | 101 |
| *BLM* | 1 | 22 | 24 | 41 | 25 | 63 |
| *CFTR* | 66 | 1943 | 72 | 113 | 138 | 2056 |
| *CLRN1* | 1 | 26 | 7 | 17 | 8 | 43 |
| *DLD* | 2 | 31 | 4 | 4 | 6 | 35 |
| *FANCC* | 3 | 45 | 23 | 36 | 26 | 81 |
| *G6PC* | 8 | 92 | 10 | 12 | 18 | 104 |
| *HEXA* | 6 | 157 | 26 | 38 | 32 | 195 |
| *IKBKAP* | 1 | 76 | 20 | 22 | 21 | 98 |
| *MCOLN1* | 2 | 36 | 8 | 10 | 10 | 46 |
| *PCDH15* | 1 | 21 | 24 | 26 | 25 | 47 |
| *SMPD1* | 4 | 50 | 19 | 25 | 23 | 75 |
| ***Total*** | ***104*** | ***2663*** | ***299*** | ***430*** | ***403*** | ***3093*** |

[1]Mutations detected by traditional carrier screening assays used by major competitors.

## Discussion

Our results show that a significant proportion of disease alleles are rare in a broad ethnic population. Most genotyping panels are based on current ACOG/ACMG screening recommendations and they tend to be accurate in limited populations, such as the Ashkenazi Jewish (AJ). Recently, extended genotyping panels have been offered that test for additional mutations, but, as we demonstrate, they still only detect a portion of the known rare disease alleles in our population, and therefore miss carriers.

"Rare" mutations may actually occur at a relatively high frequency. In clinical practice, this finding has implications for many patients.

- For individuals that are not of AJ descent being screened for the AJ disorders. Patients who are not AJ are more likely to carry a rare mutation than a common one when screened for the AJ disorders (e.g., Canavan disease).
- Carrier screening is often offered to one reproductive partner first, and to the second partner only if the first is positive. Screening the first partner with a test with a high detection rate reduces the likelihood of missing an at risk couple.
- Donors screened with a highly accurate test will have a lower residual risk. Intended parents can be more reassured that their risk of having a child with a common and severe genetic disorder is very low.

## Conclusions

Due to the vast number of pathogenic mutations detectable for each gene, NGS more comprehensively evaluates carrier status, yields higher detection rates, and reduced residual risk irrespective of patient ethnicity, resulting in fewer missed carriers .

| | |
|---|---|
| **71,070** | • Patients screened at IVF centers |
| **3,093** | • Carriers detected |
| **403** | • Distinct mutations across 15 genes (14 disorders) |
| **299** | • GSG-only mutations |
| **430 (13.9%)** | • Carriers of guideline-recommended disorders that would have been missed by other tests. |

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