Genetics in Medicine (2022) ■, 100317

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Genetics in Medicine
An Official Journal of the ACMG

www.journals.elsevier.com/genetics-in-medicine

ARTICLE

# Incidental molecular diagnoses and heterozygous risk alleles in a carrier screening cohort

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ARTICLE INFO

*Article history:*
Received 18 May 2022
Received in revised form
30 September 2022
Accepted 2 October 2022
Available online xxxx

*Keywords:*
Carrier screening
Genetic counseling
Heterozygous risk alleles
Pathogenic genotypes

ABSTRACT

**Purpose:** Expanded pan-ethnic carrier screening is an effective tool for the management of reproductive risk. However, growth in the number of conditions screened, in combination with increasingly more comprehensive test methodologies, can lead to the detection of genetic findings that may affect the health of the tested individual. The objective of this study was to investigate the frequency of pathogenic genotypes in a presumed healthy carrier screening cohort to facilitate broader discussions regarding disclosure of genetic information from carrier screening.

**Methods:** A retrospective analysis of 73,755 targeted carrier screens was performed to identify individuals with pathogenic genotypes and heterozygous risk alleles.

**Results:** In this study, we identified 79 individuals (0.11%) with pathogenic genotypes associated with moderate to profound autosomal recessive or X-linked conditions. In addition, 10 cases had chromosome X dosage abnormalities suggestive of a sex chromosome abnormality. Heterozygote risk alleles represented the majority of ancillary findings in this cohort, including 280 female carriers of *FMR1* premutation alleles, 15 heterozygous females with pathogenic DMD variants, and 174 heterozygotes with pathogenic variants in genes that may confer increased risk for somatic malignancies in the heterozygous state.

**Conclusion:** These data suggest that nearly 1% of individuals undergoing carrier screening will have a finding that may require clinical evaluation or surveillance.



## Introduction

Carrier screening for severe autosomal recessive (AR) and X-linked conditions has been systematically practiced for decades. Since its inception, carrier screening has evolved from single-gene tests targeted to ethnic groups with high carrier frequencies[1,2] to pan-ethnic multigene panels. Current practice resources published by the American College of Obstetricians and Gynecologists and the American College of Medical Genetics and Genomics (ACMG) support the practice of carrier screening for diverse populations and provide specific guidance with respect to the conditions that should be minimally included in a carrier screen.[3,4] Although carrier screening remains underutilized in individuals without presumptive risk factors,[5-8] recent studies have demonstrated

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doi: https://doi.org/10.1016/j.gim.2022.10.001

the cost-effectiveness and clinical utility for at-risk couples that proactively manage reproductive outcomes.[9,10]

Recent trends in commercial carrier screening services suggest a shift toward expanded carrier screening strategies capable of testing for hundreds of rare conditions using a combination of next-generation sequencing (NGS) and other gene-specific methodologies. Adoption of increasingly comprehensive testing strategies improves test sensitivity and yield across multiple ethnicities[11-14]; however, an unintended consequence of expanded screening is the identification of findings unrelated to the scope of the initial screen. In the context of a carrier screening panel, such findings may include the detection of a sex chromosome abnormality, variants that confer increased risk for adult-onset conditions in the heterozygous state, and clinically significant variants in the homozygous or compound heterozygous state. While identification of affected and at-risk individuals through carrier screening is predicted to be low, given that genetic conditions included on expanded carrier screening panels are typically rare in the general population, individuals with pathogenic genotypes predictive of Mendelian disease have been identified in broad population screens.[15,16]

The reporting of pathogenic genotypes and heterozygous risk alleles from carrier screening has not been specifically addressed by professional medical societies, but existing guidelines for diagnostic sequencing recommend disclosure of secondary findings for a limited group of actionable conditions.[17] The application of this standard to carrier screening is complicated, given that most findings from a carrier screen can be considered actionable because of the associated reproductive risks. Moreover, this standard does not directly examine ethical obligations to report or the potential for psychosocial harm to individuals subsequent to disclosure of secondary findings. Therefore, it is imperative to investigate the frequency and scope of unanticipated findings in carrier screening specimens to guide future discussions regarding disclosure. Herein, we report results from a retrospective analysis of 73,755 carrier screens. In accordance with previous large, population-based studies, relatively few individuals had findings consistent with a molecular diagnosis for a severe highly penetrant condition.[15,16] Conversely, a larger percentage of cases were found to harbor variants in genes that may confer increased risk for an adult-onset condition, including cancer. These results underscore the need for additional discussions surrounding consent and reporting practices related to carrier screening.

## Materials and Methods

### Case selection and review

A total of 74,839 consecutive samples submitted for testing via NGS carrier screening panels were selected for a retrospective analysis. The screening panels included a society-guided panel, an Ashkenazi Jewish panel, and a universal carrier screen (UCS) (Supplemental Table 1). Of note, only orders corresponding to the most comprehensive and most current test panel were retained for patients who had repeat testing performed, and sex-specific reproductive age ranges were used to filter out potentially inappropriate test orders. For female specimens, the reproductive age range was fixed from 15 to 49 years, whereas a range of 15 to 69 years was selected for male specimens. After removing duplicate orders and applying age cutoffs, demographic information and screening results for the remaining 73,755 specimens were reviewed, including results obtained from non-NGS components of the carrier screening panels (ie, Fragile X CGG repeat size and *SMN1* copy number). To identify individuals with pathogenic genotypes, results were reviewed for the presence of at least 2 pathogenic or likely pathogenic variants reported in a single gene associated with an AR condition, a single clinically significant variant in an X-linked gene in males, or 0 copies of the *SMN1* gene. For recessive conditions, evidence of phasing was required to confirm compound heterozygosity. Individuals suspected of having sex chromosome abnormalities were identified through chromosome X dosage analysis subsequent to the detection of full gene deletions or duplications of the *DMD* gene. Cases categorized as at risk comprised individuals with heterozygous variants in genes that conferred increased risk for adult-onset disease, such as *FMR1* premutation alleles and pathogenic variants in genes associated with cancer risk. Testing performed was in compliance with the Clinical Laboratory Improvement Amendments of 1988 federal regulations and College of American Pathologists accreditation standards. Per the United States Code of Federal Regulations for the Protection of Human Subjects, institutional review board exemption is applicable because of de-identification of the presented data (45 CFR part 46.101(b)(4)). Written informed consent was obtained from all patients by the referring physician at the time that testing was ordered.

### Race and ethnicity analysis

Analysis of race and ethnicity in this study was performed because disease prevalence and variant allele frequencies are known to vary according to ancestry. All data collected on race and ethnicity were voluntarily self-reported on test requisitions. Individuals who elected to provide information were grouped into the following categories by the investigators based on geographic divisions and shared ancestry: African or African American, African Caribbean, Ashkenazi Jewish, Asian (unspecified), Caribbean, Caribbean Indian, East Asian, Hispanic, Indigenous American, Jewish (unspecified), Middle Eastern, Sephardic Jewish, South Asian, Turkic, and White or European. Individuals who identified as more than one of the assigned categories were classified as mixed ancestry. The category of other (unspecified) was reserved for cases that self-identified as

other without providing additional information regarding their racial and ethnic background, whereas the unknown category comprised individuals who did not disclose race or ethnicity.

### NGS and bioinformatics

Genomic DNA libraries were prepared from extracted DNA using the Agilent SureSelectXT hybridization capture method. Regions of interest spanning known pathogenic variants were captured with custom-designed baits, and the libraries were sequenced on the Illumina HiSeq 2500. An internally-developed and validated workflow using the CLCbio Genomic Server v9.1.1 and Workbench software v10.1.1 (Qiagen Bioinformatics) was used for read alignment to the human genome reference GRCh37/hg19 and variant detection. Bioinformatic analysis of the NGS screening panels used in silico variant masking to selectively screen for a curated set of >9000 pathogenic and likely pathogenic variants in the targeted genes for each panel. Minimum coverage requirements included 200× mean coverage of targeted regions with >20× coverage at 99% of bases, and a minimum of 15× coverage for all designated regions of interest. Targeted regions that fell below this threshold were reflexed to a Sanger rescue pathway.

### *HBA1/HBA2* copy number detection

Common *HBA1/HBA2* deletions (Filipino, Thai, Southeast Asian, Mediterranean, alpha 3.7, alpha 4.2, HS-40 regulatory region, and alpha 20.5) and the Hb Constant Spring variant were identified by multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA P140 Probe Mix (MRC Holland). Raw data were analyzed with GeneMarker v2.7.0 (SoftGenetics). Heterozygous alpha 3.7 deletions were not subject to confirmatory testing because a large subset ($n > 1000$) of previously confirmed specimens failed to identify false-positive MLPA results. All other deletions were confirmed by repeat MLPA and/or multiplex polymerase chain reaction (PCR) and gel electrophoresis. Hb Constant Spring variants identified by MLPA were confirmed by Sanger sequencing.

### *DMD* copy number detection

Preliminary copy number calls encompassing exons of the *DMD* gene are identified by a validated, proprietary copy number variant (CNV) detection algorithm that uses normalized NGS read depth data to call deletions and duplications. Identified variants were confirmed by MLPA using the SALSA MLPA P034 and P035 probe mixes (MRC Holland). MLPA data were analyzed using GeneMarker v2.7.0.

### Fragile X CGG repeat PCR and Southern blot

*FMR1* repeat expansion was assessed for all female carrier screening cases using PCR and capillary electrophoresis. Sizing of the CGG tracts within the 5′ untranslated region of the *FMR1* gene was performed using primers that flanked the trinucleotide repeat sequences. A triplet-primed PCR using a chimeric CGG forward primer was also performed to rule out allelic dropout in individuals harboring large full mutations. The reverse primer in both reactions was labeled with 6-carboxyfluorescein to permit fluorescent detection of the PCR products. The amplified DNA was mixed with a fluorescent ladder, denatured, and loaded on an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific). ABI output files were uploaded into GeneMapper software v4.0 (Thermo Fisher Scientific) for analysis and peak calling. Specimens with expanded *FMR1* alleles were reflexed to Southern blot for confirmatory testing. Southern blot was performed by electrophoretic separation and immobilization of EcoRI- and EagI-HF–digested DNA, followed by hybridization with a StB12.3 $^{32}$P-labeled probe.

### *SMN1* and *SMN2* copy number assay

*SMN1* copy number analysis was performed by quantitative real time PCR using primers and TaqMan probes (Thermo Fisher Scientific) targeted to exon 7 of the *SMN1* gene. Each specimen was assayed in quadruplicate using different internal control sequences for data normalization. Specimens with less than 2 copies of *SMN1* were Sanger sequenced to rule out the presence of polymorphisms at primer and probe binding sites. Determination of *SMN2* copy number by digital droplet PCR was performed for specimens with zero copies of *SMN1*.

## Results

### Cohort characteristics and carrier screening panels

A total of 74,839 consecutive carrier screening orders were entered into a centralized laboratory information system dedicated to the reporting of NGS-based carrier screening tests during the time period assessed. The full data set was extracted from the laboratory information system, and a series of filtering steps were applied to these data to obtain a set of nonredundant specimens for downstream analysis. After removal of repeat orders and specimens outside of designated reproductive age ranges, a subset of 73,755 specimens remained for analysis. The final carrier screening cohort was overwhelmingly composed females ($n$ = 67,047), with males making up only 9% of cases (Figure 1A). Analysis of the cohort age distribution indicated that 70% of the female group were between the ages of

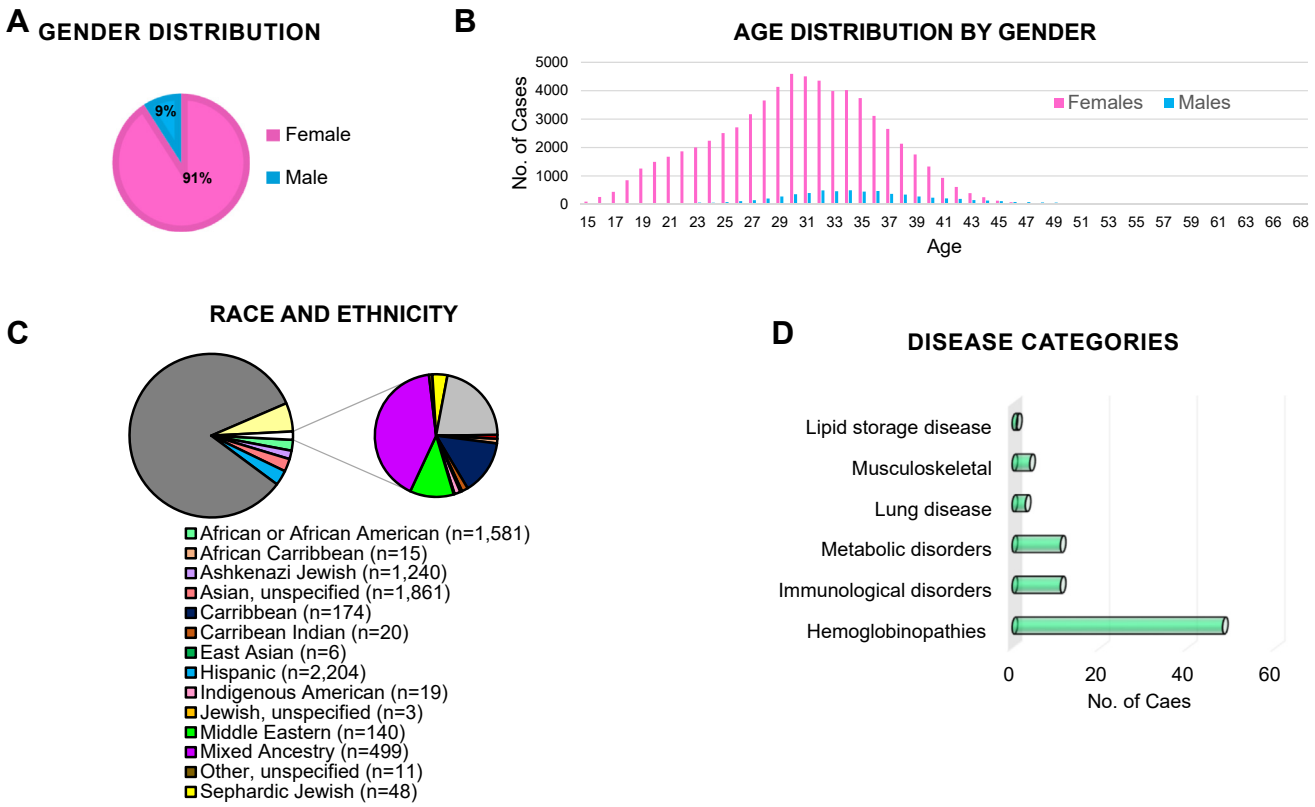


**Figure 1** **Cohort characteristics and identified disease genotypes.** A. Sex composition of the carrier screening cases. B. Age distribution of female and male cases in the cohort. C. Race and ethnic composition of the cohort. D. Case counts for presumed affected individuals by disease category.

20 and 34 years, whereas the majority of male cases were equally divided between the age ranges of 20 to 34 years and 35 to 49 years (Figure 1B). The racial and ethnic composition of the cohort was largely unknown because the majority ($n$ = 61,487; 83.3%) of tested individuals did not provide ancestral information (Figure 1C). The cases that reported race and ancestry ($n$ = 12,268) primarily comprised Africans or African Americans (12.9%), Ashkenazi Jewish (10.1%), Asians, unspecified (15.2%), and Whites or Europeans (34.0%) (Figure 1C). The rare racial and ethnic categories, including individuals with mixed ancestry, collectively accounted for 27.8% of the self-reported cases (Figure 1C; Supplemental Table 1).

Carrier screening test options included an Ashkenazi Jewish panel, a society-guided panel compliant with the minimum ACMG and American College of Obstetricians and Gynecologists recommendations, and a larger UCS (Supplemental Table 2). The majority (135/142; 93.7%) of annotated genes selected for these panels adhered to ClinGen criteria for strong to definitive association with an AR or X-linked condition[18-20] and met published criteria for moderate to profound disease symptoms.[21] The society-guided and UCS accounted for 53.7% and 41.5% of the orders, respectively (Supplemental Table 3). Notably, panel design updates were deployed during this time frame, including the addition of *HBA1* and *HBA2* deletion analysis to all NGS carrier screening panels and *DMD* sequencing with copy number analysis to the UCS. Approximately 66.1% ($n$ = 48,745) of all carrier screening cases were tested for *HBA* deletions and 63.5% ($n$ = 19,431) of the universal carrier screening cases included both genes (Supplemental Table 3).

## Pathogenic genotypes in the carrier screening cohort

To determine the frequency of individuals who receive a molecular diagnosis through carrier screening, results for the screening cohort were first reviewed for the presence of homozygous or compound heterozygous variants of known phase in AR genes. A total of 478 such cases were identified. Most cases harbored variants associated with mild or asymptomatic presentations, including 308 individuals with alpha thalassemia trait (alpha 3.7 deletion homozygotes) and 93 individuals homozygous or compound heterozygous for the Duarte galactosemia variant.[22,23] The remaining cases ($n$ = 77) were enriched for variants that cause severe Mendelian diseases, including 2 *CFTR* p.F508del homozygotes (NM_000492.3:c.1521_1523delCTT), 18 sickle cell homozygotes (*HBB* NM_000518.4:c.20A>T), and 2 cases with homozygous deletion of *SMN1* exon 7 (Tables 1 and 2). Despite the presence of pathogenic variants in these cases, many of the identified conditions have been associated with variable age of onset or intermittent symptoms, such as Wilson disease and familial Mediterranean fever. Variable

**Table 1** Homozygotes with pathogenic genotypes detected in 73,755 carrier screening cases

| Gene Symbol | Variant | Variant Classification | Race and Ethnicity (No. of Cases) | No. of Confirmed Homozygotes | % of Cases | No. of Homozygotes in the Genome Aggregation Database (v.2.1.1) | gnomAD Allele Number | gnomAD Homozygote Age Distribution | Global Minor Allele Frequency |
|---|---|---|---|---|---|---|---|---|---|
| *ALDOB* | NM_000035.3:c.1005C>G | Pathogenic | NP | 1 | 0.0033 | 0 | 280,458 | NA | 0.0001212 |
| *ATP7B* | NM_000053.2:c.2804C>T | Pathogenic | AS | 1 | 0.0029 | 0 | 280,706 | NA | 0.0001746 |
| *ATP7B* | NM_000053.2:c.2383C>T | Pathogenic | NP | 1 | 0.0029 | 0 | 249,580 | NA | 0.00002805 |
| *ATP7B* | NM_000053.2:c.3207C>A | Pathogenic | NP | 1 | 0.0029 | 0 | 280,766 | NA | 0.001019 |
| *CFTR* | NM_000492.3:c.1521_1523del | Pathogenic | NP (2) | 2 | 0.0027 | 1 | 282,630 | NR | 0.007172 |
| *CFTR* | NM_000492.3:c.3209G>A | Likely pathogenic | NP | 1 | 0.0014 | 3 | 250,926 | 35-50 (1); >50 (2) | 0.0006097 |
| *DPYD* | NM_000110.3:c.1905+1G>A | Pathogenic | NP | 1 | 0.0033 | 9 | 282,660 | 35-50 (3); >50 (3) | 0.005689 |
| *GAA* | NM_000152.3:c.-32-13T>G | Pathogenic | NP (2) | 2 | 0.0065 | 1 | 251,700 | 55-60 (1) | 0.003401 |
| *HBB* | NM_000518.4:c.19G>A | Pathogenic | AA (1), NP (4) | 5 | 0.0068 | 1 | 282,566 | 35-50 (1) | 0.001235 |
| *HBB* | NM_000518.4:c.20A>T | Pathogenic | AA (3), NP (15) | 18 | 0.0244 | 4 | 282,580 | >50 (2) | 0.004374 |
| *HBB* | NM_000518.4:c.79G>A | Pathogenic | NP (5) | 5 | 0.0068 | 1 | 282,758 | >50 (1) | 0.0002334 |
| *MEFV* | NM_000243.2:c.2177T>C | Pathogenic | AJ (2), NP (3) | 5 | 0.0146 | 9 | 282,780 | >50 (2) | 0.001983 |
| *MTTP* | NM_000253.2:c.1783C>T | Pathogenic | NP | 1 | 0.0029 | 0 | 0 | NA | NA |
| *PAH* | NM_000277.1:c.1139C>T | Pathogenic | NP | 1 | 0.0029 | 1 | 282,648 | <30 | 0.0004175 |
| *PAH* | NM_000277.1:c.442-1G>A | Pathogenic | NP | 1 | 0.0029 | 0 | 0 | NA | NA |
| *PAH* | NM_000277.1:c.1208C>T | Pathogenic | ME | 1 | 0.0029 | 0 | 282,804 | NA | 0.0005799 |
| *SMN1* | Exon 7 deletion | Pathogenic | NP (2) | 2 | 0.0027 | 0 | 0 | NA | NA |

*AA*, African or African American; *AJ*, Ashkenazi Jewish; *AS*, Asian, unspecified; *gnomAD*, Genome Aggregation Database; *ME*, Middle Eastern; *NA*, not applicable; *NP*, not provided; *NR*, not reported.

**Table 2** Genotypes of confirmed compound heterozygote cases in the carrier screening cohort

| Disease | Gene Symbol | Variant 1 | Variant 2 | No. of Compound Heterozygotes | % of Cases | Race and Ethnicity (No. of Cases) |
|---|---|---|---|---|---|---|
| Hemoglobin H disease | *HBA1/HBA2* | NM_000517.4:c.427T>C | SEA deletion | 1 | 0.0021 | NP |
| Hemoglobin SC disease | *HBB* | NM_000518.4:c.19G>A | NM_000518.4:c.20A>T | 18 | 0.0244 | AA(6), AAand HISP (1),Haitian (1), HISP (1),NP (9) |
| Sickle β-thalassemia | *HBB* | NM_000518.4:c.20A>T | NC_000011.9(NM_000518.4):c.-79A>G | 1 | 0.0014 | NP |
| Familial Mediterranean fever | *MEFV* | NM_000243.2:c.2080A>G | NM_000243.2:c.2177T>C | 4 | 0.0117 | NP (4) |
| Familial Mediterranean fever | *MEFV* | NM_000243.2:c.2082G>A | NM_000243.2:c.2177T>C | 1 | 0.0029 | Egyptian |
| Familial Mediterranean fever | *MEFV* | NM_000243.2:c.2040G>C | NM_000243.2:c.2080A>G | 1 | 0.0029 | NP |
| Phenylalanine hydroxylase deficiency | *PAH* | NM_000277.1:c.143T>C | NM_000277.1:c.165delT | 1 | 0.0029 | NP |

*AA*, African or African American; *HISP*, Hispanic; *NP*, not provided; *SEA*, Southeast Asian.

expressivity or genetic modifiers have also been reported for many of the identified conditions. Testing for the presence of modifiers is not routinely performed for most of the included panel genes; however, the 2 individuals with zero functional copies of the *SMN1* gene were subsequently shown to carry 4 copies of *SMN2*. In addition, 36% (9/25) of the beta hemoglobinopathy cases who were screened for alpha thalassemia deletions were found to be positive for *HBA1* alpha 3.7 deletions.[24] Testing for hereditary persistence of fetal hemoglobin was not performed for these cases.

Hemizygous cases with pathogenic genotypes were assessed by reviewing data for males with pathogenic variants in the X-linked genes included on the UCS (ie, *DMD*, *IDS*, *IL2RG*, *FMR1*, *OTC*, and *PDHA1*). A total of 5156 males were sequenced on the UCS, of whom 57.5% ($n$ = 2964) had testing after the addition of *DMD* to the panel. No sequence variants were identified in these 6 genes, but *DMD* hotspot deletions (exons 45-47 and exons 43-47) were identified in 2 males. Both CNVs were predicted to cause in-frame deletions, which may result in mild or subclinical phenotypes. Heterozygosity for pathogenic X-linked variants was categorically excluded from classification as a pathogenic genotype because prediction of developmental outcomes are confounded by X chromosome inactivation patterns in females. However, 5 of 67,047 (0.007%) females screened for *FMR1* expansion alleles were heterozygous for *FMR1* full mutations (>200 CGGs).

The most prevalent disease categories identified within this cohort were blood disorders and metabolic syndromes (Figure 1D). Disorders with intellectual disability as a defining feature were absent, but some of the metabolic disorders associated with the identified genotypes may present with neurologic or intellectual deficits. Comparison of the disease frequencies observed in the screening cohort with reported pan-ethnic disease frequencies indicated that some disorders, including Wilson disease and the beta hemoglobin disorders, were present at higher frequencies in our cases than the general population. In addition, the frequency of many homozygous variants was higher in the carrier screening cohort compared with the number of cases reported in the Genome Aggregation Database (gnomAD) v.2.1, particularly for *HBB* variants (Table 1).

## Sex chromosome abnormalities

Suspected sex chromosome abnormalities were initially flagged by the *DMD* copy number analysis algorithm, which performs a depth of coverage analysis of all exons of the *DMD* gene. Cases with full gene deletions or duplications were subsequently reflexed to an extended coverage analysis of additional regions of interest on the short and long arms of chromosome X to discriminate between focal CNVs and gross chromosomal abnormalities. Two males (2/2964; 0.07%) and 7 females (7/16,467; 0.04%) were found to have gains in chromosome X dosage across all regions of interest relative to other batched specimens, suggesting Klinefelter and triple X syndrome, respectively. In addition, 1 female with possible Turner syndrome/monosomy X was identified. Notably, tissue-specific mosaicism cannot be excluded due to testing of a single specimen type. With the exception of Turner syndrome, the frequency of suspected triple X and Klinefelter syndrome in this cohort approach the estimated population frequency of 0.1% for sex chromosome abnormalities.

## At-risk heterozygotes

Identification of at-risk heterozygotes is an often overlooked aspect of carrier screening because of the intentional exclusion of genes associated with dominant predisposition and adult-onset disorders. However, some commonly tested genes are known to increase risk for an allelic condition or somatic malignancy. In addition, apparently unaffected female heterozygotes with pathogenic variants in some X-linked genes may also have increased risk for delayed-onset, attenuated, or progressive disease symptoms. In this study, investigation of the frequency of at-risk heterozygotes was limited to fragile X premutation–associated conditions,[25-29] select X-linked disorders, and conditions with evidence of increased risk for cancer (eg, chromosome instability disorders). Notably, although male *FMR1* premutation carriers are also at risk for fragile X premutation disorders, *FMR1* expansion was only assessed as part of female carrier screens because of the sex-specific risk of transmitting full mutations causative for fragile X syndrome in offspring.

To identify females at risk for late-onset or attenuated X-linked disease, carrier screening results were reviewed for heterozygous cases with *FMR1* premutation alleles and pathogenic variants in *OTC*, *IDS*, *PDHA1*, and *DMD*. *FMR1* full mutations and *IL2RG* variants were not considered for inclusion because their respective phenotypes (intellectual disability and immunodeficiency) are expected to be congenital or present during childhood. Of 67,047 females who were screened for *FMR1* expansions, 280 (0.42%) carriers of premutation alleles were identified (Figure 2A). Most premutation carriers had between 55 and 70 repeats (229/280; 81.8%) (Figure 2A). Forty-three females had premutation alleles with greater than 70 CGG repeats, of which 14 carried expanded alleles with more than 90 CGG repeats. Although development of Fragile X-associated tremor/ataxia syndrome and Fragile X-associated primary ovarian insufficiency is generally correlated with higher repeat numbers, risk stratification is not possible based on the current literature on this subject. No pathogenic variants were identified in 25,422 females screened for *OTC*, *IDS*, or *PDHA1*. However, 15 female *DMD* heterozygotes at risk for cardiomyopathy were identified among the subset of female cases ($n$ = 16,467) who were screened for *DMD* sequence variants and CNVs (Supplemental Table 4). CNVs comprised 86.7% (13/15) of the detected variants, of which the majority were deletions.

The frequency of heterozygotes at risk for malignancies was assessed by filtering the screening results for individuals with pathogenic variants associated with an AR

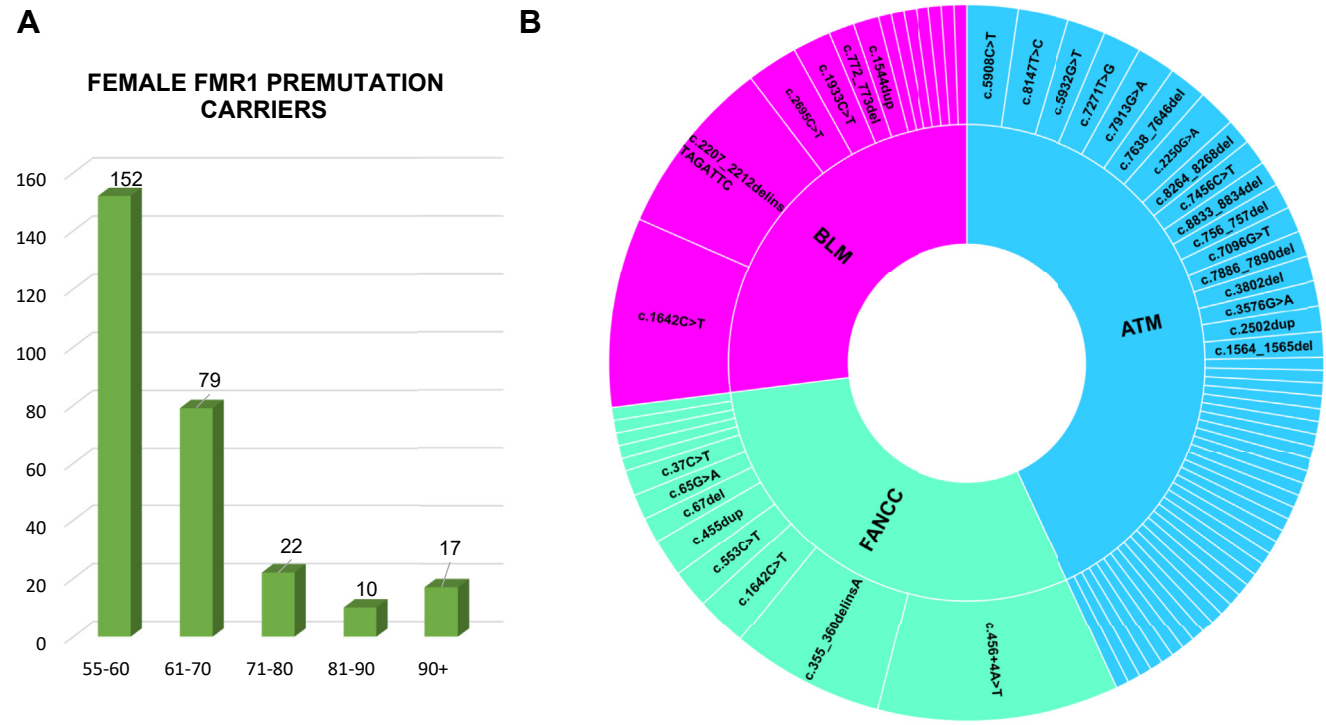


**Figure 2** **At-risk heterozygotes in the carrier screening cohort.** A. Distribution of female FMR1 premutation allele carriers by CGG repeat range. B. Hierarchical plot of cancer predisposition genes and variants identified in test specimens.

chromosome instability disorder. Seven chromosome instability disorder genes (*ATM*, *BLM*, *ERCC5*, *FANCC*, *NBN*, *XPA*, and *XPC*) are included on at least one of the carrier screening panels, and we detected 228 heterozygotes with pathogenic variants within 1 of the 7 genes. However, the majority of these genes currently have insufficient evidence to unequivocally support increased cancer risk for heterozygotes. Application of more stringent criteria for selection as a heterozygote risk gene eliminated *ERCC5*, *NBN*, *XPA*, and *XPC* from consideration. These criteria included (1) clinical guidelines that address screening of individuals with pathogenic variants in the heterozygous state, (2) at least 2 case-control studies citing enrichment of pathogenic variants in cases for any cancer, (3) evidence of a pathogenic variant segregating with disease in multiple affected family members, (4) documentation of a second hit or loss of heterozygosity in the tumor of a germline heterozygote, and (5) animal models or in vitro studies supporting increased risk in heterozygotes. The presence of clinical management guidelines was considered sufficient for inclusion (ie, *ATM)*, but genes were not excluded if increased risk was supported by case-control studies and 2 additional criteria were met. After filtering out genes with limited evidence, 174 heterozygotes with pathogenic variants in *ATM*, *FANCC*, or *BLM* remained (Figure 2B, Supplemental Table 5). The gnomAD global minor allele frequencies were not strongly associated with variant counts observed in this cohort (Supplemental Figure 1). However, 60 (33.7%) at-risk heterozygotes harbored a founder variant (Supplemental Table 5). Recently, the ACMG recommended pan-ethnic screening for 113 genes with carrier frequencies $\geq$1:200.[4] Application of these guidelines would result in the exclusion of 75 at-risk individuals with variants in *ATM* from this analysis and a 42.1% reduction in yield.

## Discussion

The reporting of secondary findings and genetic information not directly related to the indication for testing has been the subject of considerable debate within the genetics community. Generally, attitudes regarding the reporting of unanticipated findings favor disclosure when the benefits outweigh the potential for harm, particularly for actionable findings. Professional society recommendations have addressed disclosure of secondary findings for a narrow set of actionable conditions identified through large-scale genomic sequencing.[17] Although a clear distinction can be made between diagnostic and population-based screening, the majority of discoveries from carrier screens are both actionable and beneficial because the findings inform reproductive risk and may improve clinical management or mitigate the risk of severe outcomes through treatment and surveillance.

Herein, we investigated the frequency of incidental molecular diagnoses and risk alleles in a carrier screening cohort. A relatively small percentage of tested individuals received a molecular diagnosis through screening. Based on the observed genotypes, some of the affected individuals would be predicted to manifest severe symptoms of a Mendelian disease, including sickle cell disease and cystic fibrosis. However, many of the conditions identified have known modifiers of disease severity. For example, the

severity of beta hemoglobin disorders can be reduced by continued expression of fetal hemoglobin or coinheritance of alpha globin deletions which results in a reduction in the total number of abnormal hemoglobin molecules.[30] In addition, increased *SMN2* copy number can decrease spinal muscular atrophy severity because the SMN2 protein is functionally redundant with SMN1 when expressed at levels high enough to compensate for aberrant splicing of the *SMN2* gene.[31,32] The expression of disease traits can also be widely variable, even for severe Mendelian disorders without established genetic modifiers. Examples include Wilson disease, which can be predominantly hepatic or neurologic and may present in the fourth decade or later,[33] and phenylalanine hydroxylase deficiency, which can present as severe, syndromic cases of phenylketonuria or subclinical hyperphenylalaninemia.[34] Interestingly, the frequency of some pathogenic genotypes and diseases in this cohort was higher than reported in gnomAD or the general population. These discordant findings can be partially attributed to differences between the ethnic composition of the data sets, but other unknown biases may also contribute to the observed differences.

In addition to the Mendelian disease genotypes, marked deviation in chromosome X coverage, suggestive of a sex chromosome abnormality, was identified in 10 cases. Many females with triple X syndrome lack discernable phenotypes,[35] but a diagnosis of Turner syndrome or Klinefelter syndrome would be predicted to cause developmental anomalies and infertility. Consequently, reporting of patient results with an emphasis on personal risk for symptoms in addition to fetal risk may be appropriate in some instances.

Most findings identified in this cohort represented heterozygote risk alleles. At least 469 at-risk individuals were identified despite limiting the analysis to a narrow set of conditions. With respect to cancer risk, genes associated with chromosome instability disorders were selected because of the strong association with tumor development in individuals with biallelic mutations, but only 3 genes met our criteria for inclusion based on a survey of the literature. Of note, there are no existing guidelines for clinical management of *BLM* or *FANCC* heterozygotes, and guidelines for *ATM* are limited to breast cancer risk;[36] therefore, reporting cancer risk to heterozygotes requires further consideration. Additional genes on the carrier screening panels have been reported to increase risk for somatic malignancies, but these genes were excluded because of limited evidence or association with childhood cancers (eg, *CFTR* and *ELP1*, respectively). As more germline cancer studies become available, it is likely that the yield of at-risk heterozygotes on carrier screening panels will significantly increase.

Although the aim of this study was to investigate the types and frequency of pathogenic genotypes identified through carrier screens, the actual yield will vary according to the genes and methodologies selected for screening. Targeted variant screens, including the methods used herein, would predictably lead to the identification of fewer affected and at-risk individuals. A combination of full gene sequencing with high-sensitivity copy number calling and implementation of alternate methods for clinically significant regions refractory to sequencing would likely increase the yield. In addition, the utilization of algorithms capable of identifying regions of homozygosity may lead to the identification of other types of unexpected findings, including uniparental disomy and undisclosed consanguinity. However, phasing of variants would remain a challenge for short-read NGS methods that are commonly used in clinical laboratories. At least 31 cases with more than 1 pathogenic variant in the same gene were excluded from the compound heterozygote group based on the inability to phase the variants identified in these cases (Supplemental Table 6).

These studies underscore the need for additional discussion on the disclosure of disease status and ancillary risk from carrier screening assays. Although many of the variants identified within this study were reportable based on reproductive risk, risk of developing disease may not be directly communicated to patients undergoing screening despite the potential benefits to the recipient. Legal and ethical questions remain a relevant factor in the decision to disclose, as well as the patient's rights to consent to receiving information outside of fetal risk. Whether a laboratory failure to disclose rises to the level of medical malpractice or unethical behavior is an open question without easy answers given that what constitutes an actionable finding can change as the field of medicine evolves. Even currently actionable diseases or variants with strong evidence of risk, such as breast cancer risk in heterozygotes with pathogenic *ATM* variants and cardiomyopathy risk in heterozygous females with pathogenic *DMD* variants, merit consideration because the subject of disclosure should not be separated from discussions on patient consent and privacy. Current ACMG guidelines recommend discussing the risk of "manifesting" heterozygotes as part of pretest counseling,[4] but the results presented herein broaden the scope of potential findings that should be discussed with patients before screening. Future studies on patient perceptions and professional recommendations addressing these types of findings from carrier screens and other population screens will be necessary to guide laboratory screening practices in the age of the genomic medicine.

## Data Availability

The de-identified data in support of this study have been completely reported in this manuscript, primary figures, tables, and supplementary files.

## Acknowledgments

The authors would like to thank the Westborough Labcorp technologists, bioinformatics team, and genetic

coordinators, whose contributions made this study possible. Funding for this study was solely provided by Labcorp Genetics.

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## Ethics Declaration

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Per the US Federal Policy for the Protection of Human Subjects, institutional review board exemption was applicable because of the de-identification of the presented data (45 CFR part 46.101(b)(4)). Informed consent for genetic testing was required of all individuals whose data were included in this study. The contents of this manuscript have been reviewed for compliance by the Labcorp Legal department and the Department of Science and Technology.

## Conflict of Interest

J.R., L.S.R., W.X., Z.Z., H.Z., and N.L. are current employees of Labcorp Genetics, a commercial entity that receives compensation for performing genetic testing.

## Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2022.10.001) contains supplementary material, which is available to authorized users.

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