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Laboratory testing for preconception/prenatal carrier screening: A technical standard of the American College of Medical Genetics and Genomics (ACMG)

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Clinical laboratory geneticists are encouraged to document the rationale for how a particular test was designed, its intended use and its performance specifications, as well as whether or not it is in conformance with this technical standard. They also are advised to take notice of the date any particular technical standard was adopted and to consider other relevant medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures. Where individual authors are listed, the views expressed may not reflect those of authors' employers or affiliated institutions.

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ABSTRACT

Carrier screening has historically assessed a relatively small number of autosomal recessive and X-linked conditions selected based on frequency in a specific subpopulation and association with severe morbidity or mortality. Advances in genomic technologies enable simultaneous screening of individuals for several conditions. The American College of Medical Genetics and Genomics recently published a clinical practice resource that presents a framework when offering screening for autosomal recessive and X-linked conditions during pregnancy and preconception and recommends a tier-based approach when considering the number of conditions to screen for and their frequency within the US population in general. This laboratory technical standard aims to complement the practice resource and to put forth considerations for clinical laboratories and clinicians who offer preconception/prenatal carrier screening.

The Board of Directors of the American College of Medical Genetics and Genomics approved this technical standard on 26 February 2024.
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Introduction

The American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee has the mission of maintaining high technical standards for the performance and interpretation of genetic tests. In part, this is accomplished by the publication of the document "ACMG Technical Standards for Clinical Genetics Laboratories," which is now maintained online. Accordingly, the Molecular Genetics Subcommittee decided to replace the current technical standards for Ashkenazi Jewish carrier screening[1] that was due for a 5-year review in 2013 with an updated, comprehensive document that addresses laboratory considerations for preconception/prenatal carrier screening across all autosomal recessive and X-linked conditions, as a complement to the recent clinical practice resource on carrier screening.[2] The ACMG practice resource[2] considered multiple factors, including population carrier frequency, optimal panel size, and gene content, and recommended dividing carrier screening into 4 tiers. In addition, the guidance addressed the question of making panels pan-ethnic vs separating these tests by race and/or ethnicity. As a complementary document to the practice resource, this laboratory technical standard establishes the criteria for the design and validation of carrier screening tests, defines the scope and limitations of such tests, establishes the guidelines for interpreting and reporting test results, and recommends appropriate follow-up testing as applicable. This document is not intended for use as a clinical practice guideline. Disease-specific statements are intended to augment the current general ACMG Technical Standards for Clinical Genetics Laboratories. Individual laboratories are responsible for meeting the Clinical Laboratory Improvement Amendments (CLIA)/College of American Pathologists (CAP) quality assurance standards with respect to appropriate sample documentation, assay validation, general proficiency, and quality control measures.

The goal of carrier screening is to identify those at risk of transmitting a genetic disorder, traditionally focusing on autosomal recessive or X-linked conditions. Thousands of Mendelian conditions differ in both incidence and severity; however, traditional carrier screening interrogates only a subset of these conditions,[2-5] targeted toward specific ethnic populations known to be at increased risk of particular disorders, such as those of Ashkenazi Jewish descent for Tay-Sachs disease, Canavan disease, cystic fibrosis, and familial dysautonomia.[2] Recent studies have shown pan-ethnic screening for a large number of conditions more effectively identifies individuals heterozygous for a disease causing variant and at-risk couples across all races/ethnicities compared with screening that is restricted to specific races/ethnicities.[2,5-9]

Traditional carrier screening methods involved relatively simple technologies, such as polymerase-chain-reaction-based techniques, Sanger sequencing, or low-density microarrays. The earliest high-throughput carrier screening assay was a targeted genotyping panel using array-based technologies to specifically analyze hundreds to thousands of known pathogenic variants through specifically designed probes.[9] This approach was limited to a set of known pathogenic variants for each tested condition; resulting in low diagnostic sensitivity and accuracy because rare or novel pathogenic variants were undetectable.[10,11] Recent advancements in genomic technologies, such as next-generation sequencing (NGS), have enabled simultaneous screening of a large number of genes, identifying reproductive risks for dozens to hundreds of diseases, and have gained acceptance as a reasonable screening approach.[3,4,6,12] As the cost of genomic sequencing has fallen, the technology used in carrier screening has changed dramatically, allowing for low-cost, high-throughput screening with rapid turnaround times.[5,7,10,13,14] Indeed, with respect to the variant-based genotyping panel, often specific for some ethnic groups, NGS can also identify rare and novel variants.[15]

Although NGS facilitates carrier screening for a growing number of diseases simultaneously, developing a screening panel that meets the criteria to justify screening, including known positive and negative predictive values for each test, remains a challenge.[2] Uniformity across panels regarding the analytical validity and clinical utility are also a significant concern.[11,15] Difficulties in interpreting a large number of sequence variants, in cases which a majority of them are variants of uncertain significance (VUS), represent the biggest stumbling block to large-scale implementation of NGS-based carrier screening.[16] Furthermore, certain genes of high clinical importance are technically challenging to assess with NGS because of pseudogenes (*GBA* for Gaucher disease), repeat expansions (*FMR1* for fragile X syndrome), or DNA structural variations, etc.[15,17] Carrier screening using high-throughput genomic technologies are therefore highly variable in terms of panel content and technologies used (Table 1), making it difficult to compare results from different laboratories.[15]

Materials and Methods

This laboratory technical standard was informed by review of the literature and expert opinion. We consulted PubMed (search terms included: carrier screening, NGS, sanger sequencing, MLPA, polymerase chain reaction, genome sequencing, exome sequencing, and gene panels), the ACMG Technical Standards for Clinical Genetics Laboratories (2021 Revision), CLIA regulations, Online Mendelian Inheritance in Man, and Gene Reviews. When conflicting information arose within the literature, the authors used expert opinion to inform the document. Expert opinion included the authors of the document and members of the Molecular Genetics Subcommittee of the Laboratory Quality Assurance Committee. Any conflicts of interests for workgroup members are listed at the end of the paper. The Laboratory Quality Assurance Committee reviewed the

Table 1 Summary of technologies commonly used for carrier screening

	Targeted Gene Panel (TGP)		Virtual Gene Panel	
		Next-Generation Sequencing (Short Read)		
Current Testing Options	Array	Panel	Exome Sequencing	Genome Sequencing
Technology	Hybrid capture	Amplicon based/Hybrid capture	Hybrid capture	Capture free
Coverage/uniformity	NA	High (>100×)/medium	High (>100×)/medium	Medium (~30-50×)/high
Type of Variants Captured				
Single-nucleotide variants (SNV)	Yes	Yes	Yes	Yes
Small (<150 bp) insertion and deletions (indels)	Yes	Yes	Yes	Yes
Copy-number variation (CNV)	Yes (with size limitation)	Yes (with size limitation)	Yes (with size limitation)	Yes (without size limitation)
Repeat expansions	No	Limited	Limited	Emerging[a]
Regions of high homology	No	Limited	Limited	Limited
Novel variants	No	Yes	Yes	Yes
Inclusion of tier 4 genes	No	No	Yes	Yes
Data storage requirement	Low	Low	Medium	High

CNV, copy-number variant; *SNV*, single-nucleotide variant; *TGP*, targeted gene panel.
[a]The detection of repeat expansion from NGS data is still in the early stages of development.

document providing further input on the content, and a final draft was delivered to the ACMG Board of Directors for review and approval to send out for member comment. The final draft of the document was posted on the ACMG website, and an email link was sent to ACMG members inviting all to provide comments. All member comments were assessed by the authors and our recommendations were amended as deemed appropriate. Member comments and author responses were reviewed by a representative of the Laboratory Quality Assurance Committee and the ACMG Board of Directors. The final document was approved by the ACMG Board of Directors.

Design of carrier screening panels

The primary objective of a carrier screening gene panel is to maximize clinical sensitivity while minimizing the burden of analyzing genes that may not have a proven relationship with a specific disease or be too rare to contribute appreciable risk for the birth of a child with a recessive or X-linked condition. The ACMG clinical practice resource[2] on preconception and prenatal carrier screening provided guidance on optimal panel size and content. Carrier screening was divided into 4 tiers—tier 1 (cystic fibrosis, spinal muscular atrophy, and risk-based screening), tier 2 (≥1/100 carrier frequency [includes tier 1]), tier 3 (≥1/200 carrier frequency [includes tier 2]) and selected X-linked conditions, and tier 4 (<1/200 carrier frequency [includes tier 3] for which genes/conditions will vary by laboratories). ACMG recommends that all pregnant patients and those planning a pregnancy should be offered tier 3 carrier screening. This tier includes tiers 1 and 2 and comprises 97 autosomal recessive genes and 16 X-linked genes, including Duchenne muscular dystrophy (*DMD*) and Fragile X Messenger Ribonucleoprotein 1 (*FMR1*) (Table 2). Routinely screening for conditions with a carrier frequency of <1/200 adds a "diminishingly small" number of at-risk couples.[2] ACMG recommended reporting all pathogenic/likely pathogenic (P/LP) variants in the tier 3 genes.

The benefit of utilizing the tiered approach is that it facilitates communication between the patient and providers about the extent of carrier screening that may have already occurred in a prior pregnancy or fertility evaluation. Perhaps more importantly, the tier 3 category represents a list of genes and conditions that have been assessed and recommended by a panel of clinical and laboratory geneticists as having clinical relevance and utility.[2] This was accomplished by identifying those genetic disorders that have sufficient frequency within the US population and that are associated with significant impairment or need for substantial medical intervention.

The development of NGS-based approaches and their validation in routine diagnostic settings has enhanced the use of NGS-based panels for carrier screening purposes by designing targeted gene panels (TGPs), virtual gene panels (Table 1) based on a limited analysis of exome sequencing (ES), or genome sequencing (GS) data.[6,10,18-20]

TGPs

A TGP examines a curated set of genes/variants, such as ACMG-recommended tier 3 genes,[2] to evaluate the carrier

Table 2 Tier 3 genes with recommended screening methodologies

OMIM Gene Name	MANE Select and MANE Plus Clinical RefSeq Transcript IDs	OMIM Gene #	OMIM Phenotype #	Conditions	Types of Variants Present	Recommended Methodologies[a]
ABCA3	NM_001089.3	601615	610921	Surfactant Metabolism Dysfunction, Pulmonary 3	A and B	Sequencing and Del/Dup
ABCC8	NM_000352.6	600509	618857	Diabetes Mellitus, Permanent Neonatal 3	A and B	Sequencing and Del/Dup
ABCD1[b,d]	NM_000033.4	300371	300100	Adrenoleukodystrophy; ALD	A and B	Sequencing and Del/Dup
ACADM	NM_000016.6	607008	201450	Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency	A and B	Sequencing and Del/Dup
ACADVL	NM_000018.4	609575	201475	Very Long Chain Acyl-CoA Dehydrogenase Deficiency	A and B	Sequencing and Del/Dup
ACAT1	NM_000019.4	607809	203750	Alpha-Methylacetoacetic Aciduria	A and B	Sequencing and Del/Dup
AFF2[b]	NM_002025.4	300806	309548	Mental Retardation, X-Linked, Associated With Fragile Site FRAXE	C	See Table 3
AGA	NM_000027.4	613228	208400	Aspartylglucosaminuria	A and B	Sequencing and Del/Dup
AGXT	NM_000030.3	604285	259900	Hyperoxaluria, Primary Type I	A and B	Sequencing and Del/Dup
AHI1	NM_001134831.2	608894	608629	Joubert Syndrome 3	A and B	Sequencing and Del/Dup
AIRE	NM_000383.4	607358	240300	Autoimmune Polyendocrinopathy Syndrome Type I	A and B	Sequencing and Del/Dup
ALDOB	NM_000035.4	612724	229600	Hereditary Fructosuria	A and B	Sequencing and Del/Dup
ALPL	NM_000478.6	171760	146300; 241510	Hypophosphatasia, Adult; Hypophosphatasia, Childhood and Infantile	A and B	Sequencing and Del/Dup
ANO10	NM_018075.5	613726	613728	Spinocerebellar Ataxia 10	A and B	Sequencing and Del/Dup
ARSA	NM_000487.6	607574	250100	Metachromatic Leukodystrophy	A and B	Sequencing and Del/Dup
ARX[b]	NM_139058.3	300382	308350	Developmental and Epileptic Encephalopathy 1; DEE1	A, B and C	See Table 3
ASL[d]	NM_000048.4	608310	207900	Argininosuccinate Aciduria	A and B	Sequencing and Del/Dup

(continued)

Table 2 Continued

OMIM Gene Name	MANE Select and MANE Plus Clinical RefSeq Transcript IDs	OMIM Gene #	OMIM Phenotype #	Conditions	Types of Variants Present	Recommended Methodologies[a]
ASPA	NM_000049.4	608034	271900	Canavan Disease	A and B	Sequencing and Del/Dup
ATP7B	NM_000053.4	277900	606882	Wilson Disease	A and B	Sequencing and Del/Dup
BBS1	NM_024649.5	209901	209900	Bardet-Biedl Syndrome 1	A and B	Sequencing and Del/Dup
BBS2	NM_031885.5	606151	615981; 616562	Bardet-Biedl Syndrome 2; Retinitis Pigmentosa 74	A and B	Sequencing and Del/Dup
BCKDHB	NM_183050.4	248611	245600	Maple Syrup Urine Disease	A and B	Sequencing and Del/Dup
BLM	NM_000057.4	604610	210900	Bloom Syndrome	A and B	Sequencing and Del/Dup
BTD	NM_001370658.1	609019	253260	Biotinidase Deficiency	A and B	Sequencing and Del/Dup
CBS	NM_000071.3	236200	236200	Homocystinuria, B6 Responsive and Nonresponsive	A and B	Sequencing and Del/Dup
CC2D2A	NM_001378615.1	612013	612285; 612284	Joubert Syndrome 9; Meckel Syndrome 6	A and B	Sequencing and Del/Dup
CCDC88C	NM_001080414.4	611204	236600	Congenital Hydrocephalus 1	A and B	Sequencing and Del/Dup
CEP290[d]	NM_025114.4	610142	610188; 611755	Joubert Syndrome 5; Leber Congenital Amaurosis 10	A and B	Sequencing and Del/Dup
CFTR	NM_000492.4	602421	219700	Cystic Fibrosis	A and B	Sequencing and Del/Dup
CHRNE	NM_000080.4	100725	100725	Myasthenic Syndrome, Congenital, 4A, Slow-Channel; Myasthenic Syndrome, Congenital, 4B, Fast-Channel	A and B	Sequencing and Del/Dup
CLCN1	NM_000083.3	118425	255700	Congenital Myotonia, Autosomal Recessive Form	A and B	Sequencing and Del/Dup
CNGB3	NM_019098.5	605080	262300	Achromatopsia 3	A and B	Sequencing and Del/Dup
COL7A1	NM_000094.4	120120	226600	Recessive Dystrophic Epidermolysis Bullosa	A and B	Sequencing and Del/Dup

(continued)

Table 2 Continued

OMIM Gene Name	MANE Select and MANE Plus Clinical RefSeq Transcript IDs	OMIM Gene #	OMIM Phenotype #	Conditions	Types of Variants Present	Recommended Methodologies[a]
CPT2	NM_000098.3	600650	600649; 608836	Carnitine Palmitoyltransferase II Deficiency, Infantile; Carnitine Palmitoyltransferase II Deficiency, Lethal Neonatal	A and B	Sequencing and Del/Dup
CYP11A1	NM_000781.3	118485	613743	Adrenal Insufficiency, Congenital, With 46,XY Sex Reversal, Partial or Complete	A, B and C	Sequencing and Del/Dup
CYP21A2[d]	NM_000500.9	613815	201910	21-Hydroxylase Deficiency	A and B	See Table 3
CYP27A1	NM_000784.4	606530	213700	Cerebral Xanthomatosis	A and B	Sequencing and Del/Dup
CYP27B1	NM_000785.4	609506	264700	Vitamin D-Dependent Rickets, Type 1	A	Sequencing
DHCR7	NM_001360.3	602858	270400	Smith-Lemli-Opitz Syndrome	A and B	Sequencing and Del/Dup
DHDDS	NM_205861.3	608172	613861	Congenital Disorder of Glycosylation Type 1; Retinitis Pigmentosa 59	A	Sequencing
DLD	NM_000108.5	238331	246900	Dihydrolipoamide Dehydrogenase Deficiency	A	Sequencing
DMD[b]	NM_004006.3	300377	300376; 310200	Muscular Dystrophy, Becker Type, BMD; Muscular Dystrophy, Duchenne Type, DMD	A and B	Sequencing and Del/Dup
DYNC2H1	NM_001377.3; NM_001080463.2	603297	613091	Short-Rib Thoracic Dysplasia 3 With or Without Polydactyly	A and B	Sequencing and Del/Dup
ELP1	NM_003640.5	603722	223900	Familial Dysautonomia	A	Sequencing
ERCC2	NM_000400.4	126340	610756; 601675	Cerebrooculofacioskeletal Syndrome 2; Trichothiodystrophy 1, Photosensitive	A	Sequencing
EVC2	NM_147127.5	607261	225500	Ellis-van Creveld syndrome	A and B	Sequencing and Del/Dup
F8[b,d]	NM_000132.4	306700	300841	Hemophilia A	A, B and C	See Table 3
F9[b]	NM_000133.4	300746	306900	Hemophilia B	A and B	Sequencing and Del/Dup
FAH	NM_000137.4	613871	276700	Tyrosinemia Type I	A and B	Sequencing and Del/Dup

(continued)

Table 2 Continued

OMIM Gene Name	MANE Select and MANE Plus Clinical RefSeq Transcript IDs	OMIM Gene #	OMIM Phenotype #	Conditions	Types of Variants Present	Recommended Methodologies[a]
FANCC	NM_000136.3	613899	227645	Fanconi Anemia, Complementation Group C	A and B	Sequencing and Del/Dup
FKRP	NM_024301.5	606596	613153; 606612	Muscular Dystrophy-Dystroglycanopathy, Type A, 5; Muscular Dystrophy-Dystroglycanopathy, Type B, 5	A and B	Sequencing and Del/Dup
FKTN	NM_001079802.2	607440	611615; 253800	Cardiomyopathy, Dilated, 1X; Walker-Warburg Congenital Muscular Dystrophy	A, B and C	Sequencing and Del/Dup
FMO3	NM_001002294.3	136132	602079	Trimethylaminuria	A and B	Sequencing and Del/Dup
FMR1[b]	NM_002024.6	309550	300624	Fragile X Syndrome; FXS	C	See Table 3
FXN	NM_000144.5	606829	229300	Friedreich Ataxia	C	See Table 3
G6PC1	NM_000151.4	613742	232200	Glycogen Storage Disease Type Ia	A and B	Sequencing and Del/Dup
GAA	NM_000152.5	606800	232300	Glycogen Storage Disease, Type II (Pompe Disease)	A and B	Sequencing and Del/Dup
GALT	NM_000155.4	606999	230400	Galactosemia	A and B	Sequencing and Del/Dup
GBA1[d]	NM_000157.4	606463	230800; 230900	Gaucher Disease, Type I; Gaucher Disease, Type II	A and B	See Table 3
GBE1	NM_000158.4	607839	232500; 263570	Glycogen Storage Disease, Type IV; GBE1-Related Disorders	A and B	Sequencing and Del/Dup
GJB2	NM_004004.6	121011	220290; 601544	Nonsyndromic Hearing Loss Recessive 1A; Nonsyndromic Hearing Loss Dominant 3A	A and B	Sequencing and Del/Dup
GLA[b]	NM_000169.3	300644	301500	Fabry Disease	A and B	Sequencing and Del/Dup
GNPTAB	NM_024312.5	607840	252500; 252600	Mucolipidosis Type II Alpha/Beta; Mucolipidosis Type III Alpha/Beta	A and B	Sequencing and Del/Dup
GRIP1	NM_001366722.1	604597	617667	Fraser Syndrome	A	Sequencing
HBA1[d]	NM_000558.5	141800	604131	Alpha Thalassemia	A and B	See Table 3
HBA2[d]	NM_000517.6	141850	604131	Alpha Thalassemia	A and B	See Table 3
HBB	NM_000518.5	141900	603903; 613985	Sickle Cell Anemia; Beta Thalassemia	A and B	Sequencing and Del/Dup

(continued)

Table 2 Continued

OMIM Gene Name	MANE Select and MANE Plus Clinical RefSeq Transcript IDs	OMIM Gene #	OMIM Phenotype #	Conditions	Types of Variants Present	Recommended Methodologies[a]
HEXA	NM_000520.6	606869	272800	Tay-Sachs Disease	A and B	Sequencing and Del/Dup
HPS1[d]	NM_000195.5	604982	203300	Hermansky Pudlak S. 1	A and B	Sequencing and Del/Dup
HPS3	NM_032383.5	606118	614072	Hermansky Pudlak S. 3	A and B	Sequencing and Del/Dup
IDUA	NM_000203.5	252800	607014; 607015	Mucopolysaccharidosis, Ih (Hurler S); Mucopolysaccharidosis, Ih/S (Hurler-Scheie S)	A, B and C	Sequencing and Del/Dup
L1CAM[b]	NM_001278116.2	308840	307000	Hydrocephalus Due to Congenital Stenosis of Aqueduct of Sylvius; HSAS	A and B	Sequencing and Del/Dup
LRP2	NM_004525.3	600073	222448	Donnai Barrow Syndrome	A	Sequencing
MCCC2	NM_022132.5	609014	210210	3-Methylcrotonyl CoA Carboxylase 2 Deficiency	A	Sequencing
MCOLN1	NM_020533.3	605248	252650	Mucolipidosis Type IV	A and B	Sequencing and Del/Dup
MCPH1	NM_024596.5	607117	651200	Primary Microcephaly 1, Recessive	A and B	Sequencing and Del/Dup
MID1[b,d]	NM_000381.4	300552	300000	Opitz GBBB Syndrome, Type I; GBBB1	A and B	Sequencing and Del/Dup
MLC1	NM_015166.4	605908	604004	Megalencephalic Leukoencephalopathy With Subcortical Cysts	A and B	Sequencing and Del/Dup
MMACHC	NM_015506.3	609831	277400	Methylmalonic Aciduria With Homocystinuria cblC Type	A and B	Sequencing and Del/Dup
MMUT	NM_000255.4	609058	251000	Methylmalonic Aciduria-Methylmalonyl-CoA Mutase Deficiency	A and B	Sequencing and Del/Dup
MVK	NM_000431.4	251170	260920; 610377	Hyper-IgD Syndrome; Mevalonic Aciduria	A and B	Sequencing and Del/Dup
NAGA	NM_000262.3	104170	609241	Schindler Disease, Type 1; Schindler Disease, Type 3	A	Sequencing
NEB[d]	NM_001164508.2; NM_001164507.2	161650	256030	Nemaline Myopathy 2	A and B	Sequencing and Del/Dup
NPHS1	NM_004646.4	602716	256300	Finnish Congenital Nephrotic Syndrome	A and B	Sequencing and Del/Dup

(continued)

Table 2 Continued

OMIM Gene Name	MANE Select and MANE Plus Clinical RefSeq Transcript IDs	OMIM Gene #	OMIM Phenotype #	Conditions	Types of Variants Present	Recommended Methodologies[a]
NR0B1[b]	NM_000475.5	300473	300200	Adrenal Hypoplasia, Congenital	A, B and C	Sequencing and Del/Dup
OCA2	NM_000275.3	611409	203200	Oculocutaneous Albinism Brown and Type II	A and B	Sequencing and Del/Dup
OTC[b]	NM_000531.6	300461	311250	Ornithine Transcarbamylase Deficiency	A and B	Sequencing and Del/Dup
PAH	NM_000277.3	612349	261600	Phenylketonuria	A and B	Sequencing and Del/Dup
PCDH15	NM_001384140.1; NM_033056.4	605514	609533; 602083	Deafness, Autosomal Recessive 23; Usher Syndrome, Type 1F	A and B	Sequencing and Del/Dup
PKHD1	NM_138694.4	606702	263200	Autosomal Recessive Polycystic Kidney Disease	A and B	Sequencing and Del/Dup
PLP1[b]	NM_000533.5	300401	312920	Spastic Paraplegia 2, X-Linked	A and B	Sequencing and Del/Dup
PMM2	NM_000303.3	601785	212065	Carbohydrate-Deficient Glycoprotein Syndrome Type Ia	A and B	Sequencing and Del/Dup
POLG	NM_002693.3	174763	203700; 613662	Mitochondrial DNA Depletion Syndrome 4A; Mitochondrial DNA Depletion Syndrome 4B	A and B	Sequencing and Del/Dup
PRF1	NM_001083116.3	170280	603553	Hemophagocytic Lymphohistiocytosis, Familial, 2	A	Sequencing
RARS2	NM_020320.5	611524	611523	Pontocerebellar Hypoplasia Type 6	A	Sequencing
RNASEH2B	NM_024570.4	610326	610181	Aicardi Goutieres Syndrome 2	A	Sequencing
RPGR[b]	NM_001034853.2	312610	300029; 300455; 300834	Retinitis Pigmentosa 3; RP3; Retinitis Pigmentosa, X-Linked, and Sinorespiratory Infections, With or Without Deafness; Macular Degeneration, X-Linked Atrophic	A and B	Sequencing and Del/Dup
RS1[b]	NM_000330.4	300839	312700	Retinoschisis 1, X-Linked, Juvenile	A and B	Sequencing and Del/Dup
SCO2	NM_005138.3	604272	604377	Mitochondrial Complex IV Deficiency, Nuclear Type 2	A	Sequencing

(continued)

Table 2 Continued

OMIM Gene Name	MANE Select and MANE Plus Clinical RefSeq Transcript IDs	OMIM Gene #	OMIM Phenotype #	Conditions	Types of Variants Present	Recommended Methodologies[a]
SLC19A3	NM_025243.4	606152	607483	Basal Ganglia Disease, Biotin-Responsive	A and B	Sequencing and Del/Dup
SLC26A2	NM_000112.4	606718	226900; 600972	Epiphyseal Dysplasia, Multiple, 4; Achondrogenesis Ib	A	Sequencing
SLC26A4	NM_000441.2	605646	600791; 274600	Deafness Autosomal Recessive 4; Pendred Syndrome	A and B	Sequencing and Del/Dup
SLC37A4	NM_001164277[c]	602671	232220; 232240	Glycogen Storage Disease Ib; Glycogen Storage Disease Ic	A and B	Sequencing and Del/Dup
SLC6A8[b,d]	NM_005629.4	300036	300352	Cerebral Creatine Deficiency Syndrome 1	A and B	Sequencing and Del/Dup
SMN1[d]	NM_000344.4	600354	253300; 253550; 253400; 271150	Spinal Muscular Atrophy Types: I, II, III, IV	A, B and C	See Table 3
SMPD1	NM_000543.5	607608	257200; 607616	Niemann-Pick Disease, Type A; Niemann-Pick Disease, Type B	A	Sequencing
TF	NM_001063.4	190000	209300	Atransferrinemia	A	Sequencing
TMEM216	NM_001173990.3	613277	608091; 603194	Joubert Syndrome 2; Meckel Syndrome 2	A	Sequencing
TNXB[d]	NM_001365276.2	600985	606408	Ehlers-Danlos-Like Syndrome Due to Tenascin-X Deficiency	A, B and C	Sequencing and Del/Dup
TYR[d]	NM_000372.5	606933	203100; 606952	Oculocutaneous Albinism Type 1A and 1B	A and B	Sequencing and Del/Dup
USH2A	NM_206933.4	608400	276901	Usher Syndrome, Type 2A	A and B	Sequencing and Del/Dup
CLRN1	NM_174878.3	606397	276902	Usher Syndrome III	A and B	Sequencing and Del/Dup
XPC	NM_004628.5	613208	278720	Xeroderma Pigmentosum	A and B	Sequencing and Del/Dup

A, single-nucleotide variants and small insertions/deletions; *B*, exon or whole-gene deletions/duplications; *C*, repeat expansion and complex rearrangements.

[a]Sequencing (Sanger Sequencing, Next-Generation Sequencing); Del/Dup (Multiplex Ligation-Dependent Probe Amplification (MLPA), Microarray).

[b]X-linked disorders.

[c]Not a MANE select transcript.

[d]Pseudogenes or other homologous issues: *ABCD1* exon 7-10, *ASL* exon 3, *CEP290* exon 54, *F8* exon 1, *HPS1* exon 4-6, *MID1* exon 10, *NEB* exon 82-105, *SLC6A8* exon 1-13, *TNXB* exon 32-44, *TYR* exon 4-5. See Table 3 for *GBA, CYP21A2, SMN1, HBA1,* and *HBA2* genes.

status. Enriching these regions using capture or amplification methods ensures the cost to achieve appropriate coverage is reduced through efficient utilization of sequencing capacity and reduced computational and data storage requirements (Table 1).[21] The sensitivity and specificity of TGPs depend, in part, on the sequence coverage of targeted regions and the types of variants that can be detected. Panel tests can evaluate the coding and clinically relevant noncoding regions of targeted genes by NGS. However, to maximize the clinical sensitivity, laboratories should incorporate ancillary assays, such as Sanger sequencing[17] to fill in missing content or other methods to detect copy-number variants (CNVs), large structural variants (SVs), predefined complex rearrangements, or other specific variant types.

Virtual gene panels based on ES or GS

These panels examine a curated set of genes to evaluate carrier status by limiting the analysis to a set of genes from ES or GS data. ES analyzes the protein coding and adjacent intronic regions of the genome. This method involves enriching these areas through capture or amplification techniques. The depth of coverage for an exome is not uniform; therefore, the analytical sensitivity of ES may be lower than TGPs, and ancillary assays are commonly used to supplement virtual gene panels.[22] Analytical sensitivity and specificity may be compromised by inadequate coverage or quality for certain regions.[17,22] In contrast to ES and TGP, GS does not require enrichment methods before sequencing; therefore, GS produces more uniform coverage across the genome,[23] with an increased capacity to simultaneously detect single-nucleotide variants (SNVs), CNVs, repeat expansions, etc (Table 1).[23] Although coverage is more even with GS, the read depth is generally lower than TGP and ES and may therefore reduce the analytical sensitivity and specificity. The cost of data generation and storage is higher for GS than for ES.[21] The advantages of using GS over ES for virtual gene panels for carrier screening are the detection of pathogenic variants in noncoding regions, more uniform coverage of the genome, and better assessment of CNVs, which include autosomal recessive genes.[13] Using ES or GS also has the advantage of expanding the virtual gene panels by adding any number of additional genes at a later date based on ACMG guidelines, such as tier 4 genes (Table 1).

Technical considerations in the development of carrier screening panels

NGS has the ability to generate high-quality sequence data for various applications in the clinical laboratory, ranging from clinically relevant targeted panels to all the genes in the genome, thereby overcoming scalability obstacles for DNA sequencing. Nevertheless, NGS also presents new technical challenges that must be understood and addressed. Once a laboratory defines a carrier screening panel based on ACMG recommendations, technical considerations including the strengths and limitations of their chosen methodology must be evaluated. Laboratories must recognize the complex characteristics of certain genes that may complicate testing or interpretation of the pathogenic variants and when ancillary technologies may be needed to cover the spectrum of pathogenic variants of a particular disorder adequately. Additionally, the performance of different NGS methodologies may vary for genes of specific interest; therefore, the performance of the specific methodology must be evaluated and validated by the testing laboratory as outlined by ACMG.[17]

Detection of different variant types

When developing a test, it is important to consider the types of variants that may be identified in the genes or regions of the genome being analyzed. Specialized bioinformatic pipelines and highly reproducible, uniform data are necessary for detecting CNVs, genomic rearrangements, and repeat expansions through NGS. Identifying sequence characteristics that may complicate testing or interpretation is crucial, as is knowing when ancillary technologies may be necessary to adequately cover the full range of pathogenic variants (Table 1 and see Regions with technical difficulties and Ancillary technologies). The types of common P/LP variants along with the recommended testing technologies for the ACMG tier 3 genes are listed in Table 2. Tier 3 genes that encompass regions with technical difficulties or require ancillary testing technologies are listed separately in Table 3.

Regions with technical difficulties

Currently, NGS methodologies, especially short reads, are limited in detecting certain kinds of variants, such as repeat expansions and highly homologous genomic loci.[33] Genomic regions that are highly homologous or have low complexity, as well as regions that are repetitive or hypervariable, may lead to reduced or biased accuracy in the sequence alignment and variant calling. The use of hybridization-based enrichment methods in TGP and ES cannot completely prevent the capture of homologous regions of targeted genes. Moreover, the short length of NGS sequence reads generated by short-read technology can cause false-positive or false-negative variant calls if the reads are inaccurately aligned to a homologous region.[21] Resources annotating many known regions with high homology have been created.[34] Examples include *SMN1*, associated with spinal muscular atrophy (deletions in a homologous region)[35] and *FMR1* associated with fragile X syndrome (repeat expansion).[17,36] For pseudogene families, such as *CYP21A2*, associated with congenital adrenal hyperplasia,[30] and *GBA* associated with Gaucher disease, the existence of a highly homologous pseudogene presents technical challenges.[17] Because technically challenging genes are part of the recommended ACMG tier 3 genes (Table 2), proper ancillary methodology may be required (Table 3 and see Ancillary technologies). Methodological advances, such as analysis for repeat expansions,[24-26] small

Table 3 List of challenging tier 3 genes with available screening and ancillary methodologies

Gene	Detection Challenges	Ancillary Technologies	Sequencing Technologies
FMR1	Repeat Expansion (CGG repeat expansion in the 5′ UTR)	Triplet-primed PCR, Southern blot	NGS with custom caller[24-28]
FXN	Repeat Expansion (GAA repeat expansion in intron 1)	Triplet-primed PCR	NGS with custom caller[24,25,27,28]
ARX	Repeat Expansion (GCN, Polyalanine repeat expansions in exon 2)	Sanger sequencing	NGS with custom caller[25-27]
AFF2	Repeat Expansion (CCG repeats at 5′ UTR)	Triplet-primed PCR	NGS with custom caller[25,27]
GBA	Pseudogene (gene conversion between *GBA* and *GBAP*)	Long-range PCR & Sanger sequencing	NGS with custom caller[15,26,29]
CYP21A2	Pseudogene (gene conversion between *CYP21A2* and *CYP21A1P*)	Long-range PCR & Sanger sequencing and MLPA	NGS with custom caller[15,26,30]
SMN1	Homology (*SMN1* and *SMN2*)	qPCR or MLPA	NGS with custom caller[26,31]
HBA1/2	Homology (*HBA1* and *HBA2*)	qPCR or MLPA	NGS with custom caller[15,26]
F8	Inversion (Intron 22 and intron 1 inversions)	Long-range PCR, inverse PCR, PCR-based "inverse shifting" procedure	NGS[32]

NGS includes TGP, ES, and GS with short-read or long-read sequencing.

MLPA, multiplex ligation-dependent probe amplification; *NGS*, next-generation sequencing; *PCR*, polymerase chain reaction; *qPCR*, quantitative polymerase chain reaction.

deletions in a highly repetitive region,[26,31] or pseudogene families[15,26,29] in short-read NGS data, may overcome these limitations for some types of variants. Long-read sequencing technology outperforms short-read sequencing technology in the detection of repeat expansions and variants in regions of high homology containing clinically important genes.[21,37]

Reference genome and transcripts

The choice of the human reference genome and transcripts for mapping and variant calling has a direct impact on testing accuracy. Clinical laboratories primarily use GRCh37/hg19 or GRCh38/hg38 human reference genome. The current "gold standard" human genome reference assemblies curated by the Genome Reference Consortium (GRC) are GRCh37, originally released in 2009 and periodically updated until 2013 when its successor GRCh38 was published. Although many errors in the human genome sequence were corrected with the update from GRCh37 to GRCh38, some issues remain or were newly introduced, such as with Cystathionine beta-synthase (*CBS*), a gene associated with homocystinuria and thrombosis (MIM 236200). The introduction of additional reference sequences in GRCh38 for cystathionine beta-synthase like (*CBSL*), a region of chromosome 21, which contains a high-percent identity with *CBS*, resulted in multi-mapped reads when aligned against GRCh38 but not GRCh37, and this discrepancy at the *CBS* locus does affect the evaluation of carrier status for pathogenic variants because the majority of the variants in these genes could only be called on GRCh37.[38] Laboratories should consider these types of issues when using either GRCh37 or GRCh38 and may wish to use appropriately modified reference genome assemblies such as those available for GRCh38.[37]

Many genes have multiple transcripts with alternative exons. Use of the clinically relevant transcript is critical for accurate variant calling and its predicted effect on the gene/protein. The National Center for Biotechnology Information (NCBI) and Ensembl/GENCODE created consensus transcripts through the Matched Annotation by NCBI and EMBL-EBI (MANE) project for all genes without technical limitations (eg, genome assembly errors), and we recommend the use of MANE Select and MANE Plus Clinical transcripts, as well as reported pathogenic and likely pathogenic variants in ClinVar as noted below, for defining the coding and noncoding regions that must be interrogated.[39,40]

Intronic variants

Incorporating intronic region analysis to detect known pathogenic intronic variants is critical in increasing the clinical sensitivity of NGS-based carrier screening panels. Although GS covers intronic regions, most TGP and ES assays only interrogate variants within ±20 bases of the exon-intron junctions. As such, laboratories need to be aware of any intronic pathogenic variants beyond ±20 bases in the genes being analyzed and ensure sufficient coverage to detect those variants. The ClinVar database is a reasonable resource to identify known pathogenic variation outside these regions that should be interrogated.

CNVs and other SVs

The analysis of CNVs and other SVs using NGS data is still evolving, with available algorithms varying in their sensitivity and specificity.[17,41,42] The false-positive rate for CNV analysis using NGS data can be high and is often influenced by factors such as the depth and uniformity of coverage, as well as the size of the CNV or type of other SV and the sequence itself. This is particularly relevant for small, intragenic exon-level deletions and duplications, in which the false-positive rate may be higher.[17,41,42] GS data have lower false-positive and false-negative CNV detection rates compared with capture of amplification-based enrichment

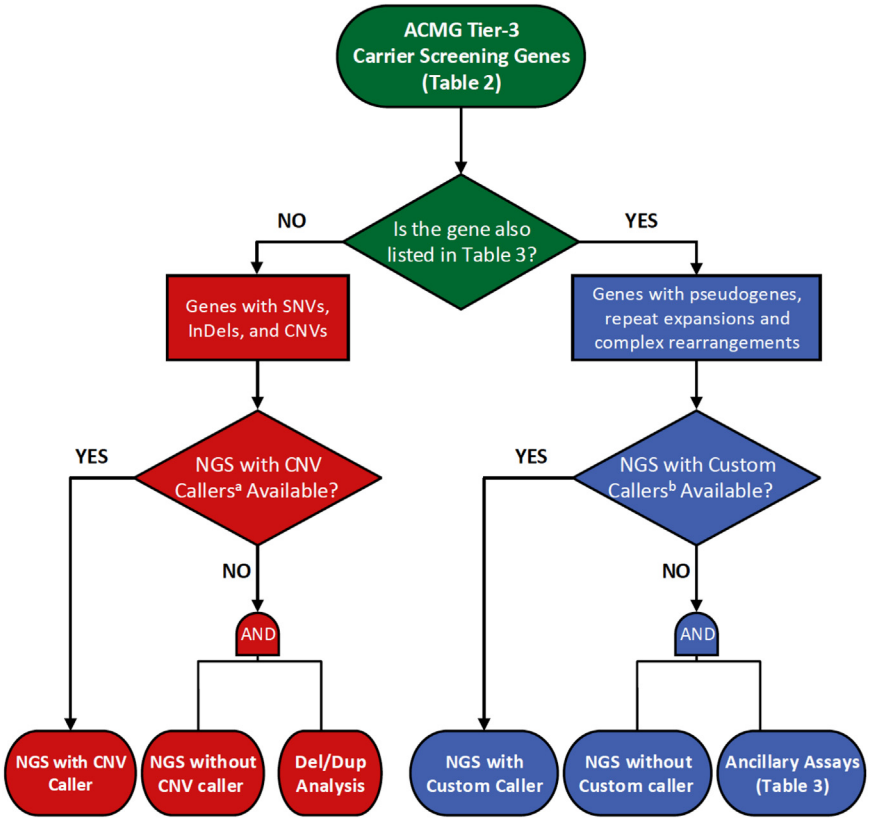

Figure 1 Recommended decision tree for selection of laboratory techniques used in tier 3 carrier screening. Based on technological availability, testing laboratories should select the appropriate assays for genes with SNVs and CNVs (red), and for genes listed in Table 3 (blue). A. Testing laboratories must ensure to detect all CNVs and Indels (>25 bp) using an NGS-based caller. B. Testing laboratories must be aware that many custom callers for genes listed in Table 3 are limited to ES or GS. In the absence of custom caller, use of ancillary assays is recommended. CNV, copy-number variant; ES, exome sequencing; GS, genome sequencing; NGS, next-generation sequencing; SNV, single-nucleotide variant.

methods, such as TGP or ES.[41,42] To ensure accurate CNV analysis and capture of other known SVs using NGS data, careful validation experiments must be performed to determine the size of CNVs below which accuracy is compromised and whether specific known SVs (eg, F8 inversion) can be detected. If necessary, confirmatory testing using orthogonal methods may also be required to validate the results. Recently, a "points to consider" document in the detection of germline structural variants using NGS has been outlined by ACMG.[42] Reports regarding CNV detection must be explicit about any limitations and clearly specify the size of CNVs (expressed in terms of the number of consecutive exons or nucleotide length) that can be accurately detected.

Ancillary technologies

Clinically relevant genomic regions that cannot be assayed reliably by NGS[33] (eg, areas with homology, low complexity, and repeats) should be considered for testing by ancillary assays. TGP that includes these areas should include appropriate additional methodologies to maximize the clinical sensitivity of the test (Table 3). Sanger sequencing can be used to fill in areas that NGS coverage or quality is insufficient to call variants confidently but may also be limited by inherent sequencing difficulties.[33] For ES/GS tests, complete coverage is not expected but laboratories should not advertise compatibility with ACMG tier 3 recommendations unless testing uses additional methodologies and/or analytical algorithms to address disease-relevant regions and cover variant types highlighted in Table 2 as needed on a gene by gene basis.

Together, this workgroup recommends an algorithm for selection of techniques used in the testing laboratories for carrier screening (Figure 1). Type of variants and available techniques in each laboratory are the important factors to select the most effective testing approach.

Validation of carrier screening panels

Once test content, assay conditions, and pipeline configurations have been established per ACMG guidelines,[21] the laboratory should have a validation plan prepared and executed from start to finish on all permissible sample types. Laboratories offering carrier screening in the US must meet

the CLIA/CAP requirements for qualification as a clinical testing laboratory. Assay performance characteristics including analytical sensitivity and specificity, accuracy, and precision should meet thresholds predetermined in the validation plan per professional guidelines.[21,43-45] Analytical sensitivity and specificity characterize the genetic assay performance by the proportion of results that are correctly classified as positive and negative, respectively.[46] The first test developed by a laboratory generally requires a more comprehensive validation than subsequent tests developed on the same platform using the same basic bioinformatics pipeline design. In practice, this may entail sequencing a larger number of samples in order to test a sufficient number of each variant type.[21]

Sample type used in testing

Performance data across tests using the same platform can be combined to establish a cumulative "platform" performance. By maximizing the number and types of variants tested across a broad range of genomic regions across all acceptable specimen types, confidence intervals can be established. Note that current "truth" sets of a few samples (eg, National Institute of Standards and Technology Genome in a Bottle samples)[37] often encompass most of these variant types across the genome and are recommended to be included. Importantly, they are a renewable resource that can aid in monitoring test performance over time and after modifications.[21]

Variant types evaluated in testing

For carrier screening, the analytical and clinical sensitivity and specificity depend on the testing method and the type of platform used. The large size of NGS tests make validation of every base impossible. However, validation may focus on the extrapolation of performance parameters for discovery of SNVs and indels within the boundaries of the established regions. Because performance of all specific events cannot be predicted, testing a variety of events (eg, type, size, and position in captured region) across different genes or regions of interest is important. The spectrum of genomic variation for carrier screening includes types of variation that may fall outside of those covered by NGS testing (Table 1). Ancillary methods should be validated and utilized to capture these (see Ancillary technologies). Otherwise, the test report should note a limitation that ancillary methods are not used, and the test cannot be claimed to cover ACMG tier 3 content. Issues related to accurate sequencing of highly homologous regions should be addressed when 1 or more genes within the test have known pseudogenes or other homologous loci. If high clinical sensitivity is based on the ascertainment of particular common pathogenic variants, these should be included in the validation. Performing genetic testing with a higher number of variants or genes increases the potential for errors, but the CLIA validation process is designed to mitigate this concern.[43]

Bioinformatics pipelines

Availability of new bioinformatic pipelines has improved the analytical sensitivity and specificity of NGS for detection of variants. Bioinformatic pipelines used in carrier screening must be validated for analysis of different types of specimens and variants according to professional guidelines established by organizations such as ACMG,[21] CAP,[44] and the Clinical Laboratory Standards Institute.[47] Validation of bioinformatics pipelines should include data from actual samples but could be supplemented with analysis of in silico data files that harbor various types of variants. Once validated, it is acceptable to use in silico analysis of the previous data for minor pipeline updates, such as security patches, with no impactful changes.[48]

Clinical validity

Clinical sensitivity and specificity are functions of how often individuals are identified as true heterozygous for a disease causing variant or not, respectively, by the genomic assay. Both analytical and clinical validity are of importance to the patient because they are incorporated into the determination of positive and negative predictive values, characteristics that are of immediate relevance for an individual receiving carrier screening results. NGS-based assays and ancillary methods are likely to have high clinical sensitivity for carrier screening. In contrast, the use of published literature and databases, as well as predictive algorithms to determine variant impact, have a lower clinical specificity (higher false-positive rate) in that variants may be erroneously called as pathogenic or likely pathogenic. Additionally, factors such as allelic and locus heterogeneity may potentially affect the clinical specificity of carrier screening.

Report elements in carrier screening

Reporting of variants

Laboratories are expected to report only those variants that are classified as pathogenic (>99% certainty) or likely pathogenic (>90% certainty) based on the current ACMG/AMP variant classification guideline.[49] However, there are exceptions leading to instances in which a VUS is reported. An example of when a VUS may be reported is when 1 member of a couple is already known to carry a pathogenic or likely pathogenic variant, and a gene-specific comprehensive sequencing approach is requested to screen the second member of the couple, after appropriate counseling. In such cases, a VUS may be identified during testing and reported in the results. This scenario may be especially important in cases that screening is performed and there is an ongoing pregnancy.

Residual risk and detection rate

An individual's residual risk to be heterozygous for a disease-causing variant after having a negative screening test can be calculated.[2] However, when carrier screening is performed by simultaneously testing multiple variants

within multiple genes for rare conditions, it may be challenging to determine the carrier frequency and detection rate for each condition being screened. Moreover, data sets used to establish carrier frequency can evolve when genomic data from new populations become available.[50] Finally, residual risk calculation is mostly based on the patient's self-identified ethnicity, which may not be a correct reflection of genomic ancestry.[51] ACMG cautions that providing a precise residual risk after carrier screening, which involves the simultaneous analysis of multiple rare or uncommon variants within genes, may not be practical.[2] Rather than providing a precise residual risk, patients should be informed that a negative screening test does not completely eliminate the possibility of being a heterozygote for any particular condition (ie, gene variant), although the risk is significantly reduced. They should also be informed that not all variants are reported, such as VUS. In cases of targeted panels that screen common P/LP variants for certain genes, patients should be notified about a minimal risk of being a heterozygote for a novel P/LP variant within the same gene.

Reproductive risk

When reporting positive results from carrier screening, the report should include a brief clinical description of the disorder, including its penetrance if known and variability in expression if understood. It may be appropriate to provide information about genotype-phenotype correlations in the report, along with any relevant limitations because these correlations may not always apply to an individual even if they are meaningful in a population. A statement about reproductive risk should be included when a heterozygote is identified.

We encourage laboratories to include a general statement regarding reproductive risk under both circumstances, (1) when a heterozygote is identified and (2) when a heterozygote is not identified. When a heterozygote is identified, the statement regarding reproductive risk may include the following components. The likelihood of an affected child requires that the child receives (1) the allele from the heterozygous parent identified and (2) a second allele from the reproductive partner. However, offspring with 2 alleles considered to be pathogenic or likely pathogenic may in some cases either not manifest the condition (reduced penetrance) or manifest a condition in a variable way (variable expressivity). These circumstances are known to occur more often for specific genes and variants. However, they can occur when less information is known about a specific gene or variant. For these reasons, genetic counseling is recommended when an individual or family is at risk.

The statement regarding residual risk may include the following components when a heterozygote is not identified for any tested condition. Residual risk represents the risk that remains after a negative screening test result. High-throughput, pan-ethnic carrier screening poses difficulties in stating the precise residual risk for many conditions simultaneously. When a patient tests negative for any condition, it is important to recognize that the risk of carrying a clinically important variant is never zero. Therefore, before testing, patients should understand that a negative test result does not eliminate the possibility of a genetic condition in their offspring, even for those conditions for which genetic screening is included. However, patients should understand that screening reduces the identifiable risk for screened conditions by orders of magnitude.

Reporting test limitations

Transparency is imperative when performing carrier screening so that ordering providers know what the screening assay includes and what it does not. As a minimum requirement, any low-coverage or nontargeted exonic regions must be listed on the report or a summary provided, along with specific instructions on how to obtain more detailed information. When reporting results from a carrier screening panel, the methodologies including any ancillary methodologies used and test limitations must be clearly delineated. The laboratory is responsible for identifying any regions or exons that perform poorly or inconsistently during test validation and providing a disclaimer or a limitations section in the report, either in summary or in detail. In addition to identifying regions or exons that perform poorly during test validation, any regions or genes that were specifically excluded from testing because of technical issues, such as the presence of pseudogenes or repeat expansions, should be specified in the report. The report should also explain in detail any technical limitations for the detection of large deletions/duplications and noncoding variants to ensure that the clinician and patient are fully informed of any potential limitations of the test.

Conclusion

A wide array of high-throughput carrier screening assays for a large number of genes, identifying reproductive risks for dozens to hundreds of diseases, are now available based on microarray or NGS technologies. However, along with the capability to produce high-quality sequence data, NGS also brings new technical challenges that must be appreciated and addressed. This document provides technical guidelines for ACMG tier 3 genes, ensuring the achievement of maximum clinical sensitivity, specificity, and validity. Although key aspects of the clinical implementation of carrier screening technologies have been addressed, laboratories are ultimately responsible for the design, validation, data generation, interpretation, and reporting of carrier screening gene panels as clinical assays. Laboratories must consider the effectiveness of the assays and either augment NGS testing using ancillary assays or disclose the limitations of the methodology given the design of the gene panel. Clearly (Tables 1 and 2), sequencing technology is a core methodology required to address the large number of genes recommended for prenatal carrier screening. High throughput combined with highly reliable results require laboratories to implement sequencing technology.

The ACMG recognizes the requirement of multiple methods to reliably address the large number of genes recommended for carrier screening will create opportunities for (1) laboratories to innovate in an effort to contain the costs of screening, (2) health care systems to analyze the benefits of high-throughput, high reliability prenatal screening for common Mendelian conditions and the cost benefit of this information across a lifetime in order to enhance reimbursement for this screening, and (3) electronic medical record systems to innovate to make test results readily available in an effort to further reduce costs of repetitive screening.

Laboratories should not advertise compatibility with ACMG tier 3 recommendations unless all genes and known variant types are analyzed and interpreted with approaches recommended here. Effective implementation of the ACMG-recommended tiered carrier screening approach[2] for equitable testing requires an ongoing dialog among those already engaged in this pursuit, those determining how to become involved in this rapidly changing paradigm of carrier screening, and those who will be responsible for ordering and communicating carrier screening results to patients.

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Conflict of Interest

Saurav Guha, Mahmoud Aarabi, Marina DiStefano, and Erin Wakeling are directors of molecular testing laboratories that offer carrier screening. Honey V. Reddi is a Consultant Director for Biofidelity Inc. Jeffrey S. Dungan is a member of the Advisory Board for Informed DNA and Medical Codirector at Insight Medical Genetics, which provides genetic laboratory services. All other authors declare no conflicts of interest.

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