Early clinical laboratory experience with a 526-gene expanded carrier screening panel

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Summary

We present here a retrospective pilot analysis of a cohort of 613 consecutive individuals tested using a novel, 526-gene expanded carrier screening panel.

Rationale for design of the panel

Carrier screening is typically performed during the preconception or prenatal period to assist couples in making informed reproductive decisions. Professional societies have issued statements in support of expanded carrier screening, along with guidance for the responsible development of carrier screening panels.[1,2] In 2015, the American College of Medical Genetics and Genomics (ACMG), the American College of Obstetricians and Gynecologists (ACOG), the Society for Maternal Fetal Medicine (SMFM), and the National Society of Genetic Counselors (NSGC), provided guidance regarding disorders to consider including in expanded carrier screening panels: disorders that are autosomal recessive or X-linked; associated with cognitive disability or the need for surgical or medical intervention; have an effect on quality of life; or offer improved perinatal outcomes when identified prior to birth.[1]

The next generation sequencing-based, 526-gene carrier screen panel designed at Labcorp includes disorders that are recommended in ACMG and ACOG guidelines, and/or meet the guidance provided by the ACMG, ACOG, SMFM, and the NSGC.[1] Disorders are preferentially included if options are available for early management and therapy, and if relevant within or across diverse ethnic groups. The panel is designed in a novel way, by including all relevant autosomal and X-linked genes associated with each disorder present on the panel.

Methods

Next-generation sequencing (NGS)

Genomic regions of interest include the entire coding region and flanking noncoding regions and are captured using custom capture baits from Twist Bioscience. Sequencing reads from the Illumina® next generation sequencing platform are aligned with the human genome reference GRCh37/hg19 build. A minimum of 99% of bases are covered at >15X. Regions with known pathogenic variants that are outside the coding and flanking noncoding regions are sequenced in a targeted manner. Analytical sensitivity is estimated to be >99% for single nucleotide variants, >97% for insertions/deletions less than six base pairs, and >95% for copy number variants. Orthogonal confirmatory studies are performed in accordance with regulatory guidelines. All technical and performance characteristics were internally validated following guidelines set forth by the College of American Pathologists (CAP).

Alternate technologies for genes not assessed by NGS

1. For spinal muscular atrophy testing, the copy number of exon 7 of the *SMN1* gene is assessed using a quantitative PCR assay. When no copies of *SMN1* exon 7 are detected, *SMN2* exon 7 copy number is assessed and reported. When two copies of *SMN1* exon 7 are detected, the data are assessed for the presence of the c.*3+80T>G variant associated with "silent carrier" risk.
2. Copy number analysis for congenital adrenal hyperplasia and alpha thalassemia assesses common variants and deletions in the *CYP21A2* gene, and the *HBA1* and *HBA2* gene cluster, respectively.
3. For fragile X syndrome testing, repeat-primed PCR is used to detect the number of CGG repeats on each allele of the *FMR1* gene. In females, alleles between 55 and 90 repeats are assessed by a PCR assay to determine the number and position of AGG interruptions within the CGG repeats.

Variant classification

Variants are classified by an in-house variant classification protocol that is traceable, and in accordance with guidelines from the American College of Medical Genetics and Genomics (ACMG). Classification uses an algorithmically-weighted assessment of several components, including: predicted functional impact determined by *in silico* analysis; prevalence of the variant in the unaffected (general) population; segregation in affected individuals or families published in peer-reviewed literature; co-occurrence with other deleterious variants; and data from functional studies. For details about the variant classification algorithm, see Labcorp's variant classification summary in ClinVar at https://www.ncbi.nlm.nih.gov/clinvar/submitters/500026/.

Carrier rates and determination of residual risks

A comprehensive approach to determining carrier frequencies is used, based on data derived from the public databases gnomAD and ClinVar. Detection rates are calculated based on validation data, variant types, and gnomAD data, and residual risks are calculated using Bayes' theorem.

Reportable variants and positivity rates

Carrier detection (positivity) rates are calculated as the percentage of individuals heterozygous for a pathogenic or likely pathogenic variant in a particular gene, compared with the total number of individuals tested. Positive results from the 526-gene panel were compared with positive results from a 141-gene panel that contains a subset of the genes in the 526-gene panel. Positivity rates were compared using a z-score calculator for two population proportions.

Results

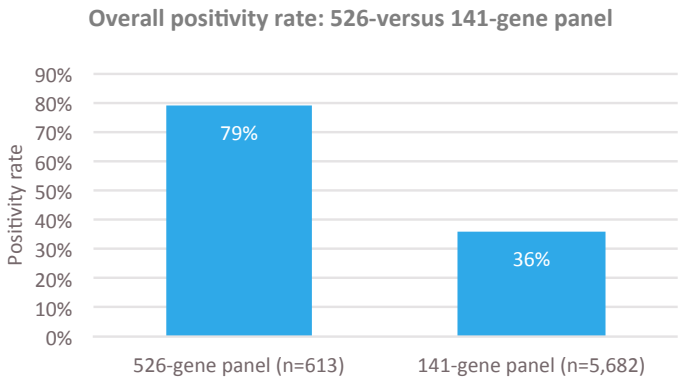
Number of carriers identified by screening with a 526-gene panel

Among 613 individuals, 79% (n=483) were carriers of at least one disorder on the panel. Of the 483 carriers, 46% (n=221) were carriers of one disorder, 34% (n=164) of two disorders, 14% (n=66) of three disorders, 5% (n=24) of four disorders, and 1% (n=8) of five or more disorders.

Carrier screening result	Proportion of individuals with this result
Negative	21%
Carrier of 1 disorder	46%
Carrier of 2 disorders	34%
Carrier of 3 disorders	14%
Carrier of 4 disorders	5%
Carrier of 5+ disorders	1%

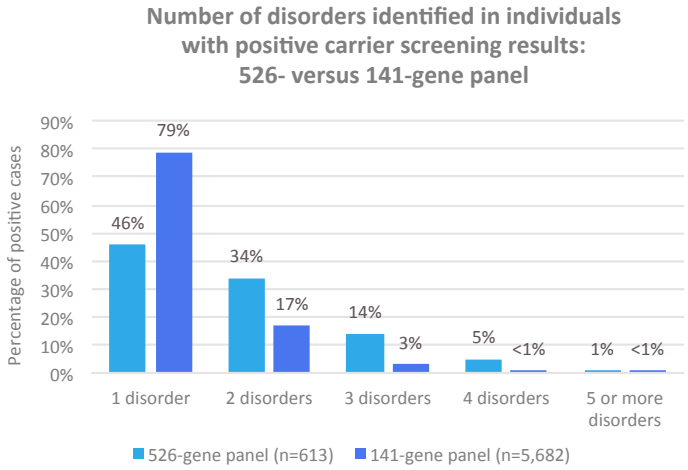
Comparison of the 526-gene and 141-gene panels

The 526-gene panel positivity rate was significantly higher than the positivity rate of the 141-gene panel (79% vs. 36%, p<.001).



Note: Following data analysis, three additional genes (*HBA1*, *HBA2*, and *DMD*) were added to the 141-gene panel, bringing the total gene count to 144. These additional genes are expected to add to the positivity rate of the panel. The combined positivity rate of *HBA1* and *HBA2* was previously reported as ~9.3%.[3] The estimated positivity rate for the *DMD* gene is <0.1% in a carrier screening population.[4]

More individuals were identified as carriers of two or more disorders from the 526-gene panel compared with the 141-gene panel (54% vs. 21%, p<.001).



Analysis of the most common disorders with positive results on the 526-gene panel

Carriers of alpha-thalassemia were most frequently detected after screening with the 526-gene panel, followed by carriers of galactosemia and biotinidase deficiency.

Most common disorders with positive results	Gene
Alpha-thalassemia	*HBA1/HBA2*
Galactosemia	*GALT*
Biotinidase deficiency	*BTD*
Cystic fibrosis	*CFTR*
GJB2-associated hearing loss	*GJB2*
Spinal muscular atrophy	*SMN1*
Beta-hemoglobinopathies	*HBB*
Familial Mediterranean fever	*MEFV*
Phenylalanine hydroxylase deficiency, including PKU	*PAH*

Note: Positive results include the variants associated with Duarte variant galactosemia (c.-119_-116delGTCA) and partial biotinidase deficiency (c.1330G>C). Patient reports with positive results for these two variants include information regarding the mild nature of these findings.

Discussion

Although individually rare, the disorders included on the 526-gene expanded carrier screening panel collectively contribute to increased carrier detection, as demonstrated by an overall 79% positivity rate. Compared with a smaller 141-gene panel that contains a subset of genes from the 526-gene panel, we show that the larger panel identifies more carriers overall, and identifies more carriers of multiple disorders. Detecting carriers of these disorders may allow for more informed reproductive decision-making and/or appropriate postnatal management, supporting the clinical utility of expanded carrier screen panels.[1]

We support the recommendation that all individuals identified as carriers of autosomal recessive disorders should be offered screening for their reproductive partners. Additionally, genetic counseling should be available to patients found to be at risk for having affected offspring. Furthermore, we support the ACOG Committee Opinion that screened conditions should be able to be diagnosed prenatally.[2] As such, all genes included in the Labcorp expanded carrier screening panels have been validated for prenatal diagnosis.

References
1. Edwards J, Feldman G, Goldberg J, Gregg A, Norton M, Rose N et al. Expanded Carrier Screening in Reproductive Medicine—Points to Consider. *Obstet Gynecol*. 2015; 125(3):653-662.
2. The American College of Obstetricians and Gynecologists, Committee on Genetics. Committee Opinion No. 690: Carrier Screening in the Age of Genomic Medicine. *Obstet Gynecol*. 2017; 129(3):e35-e40.
3. Walker A, Robinson M, Smith J, et al. An automated α-globin region genotyping tool for the detection of copy number variants by MLPA in a clinical setting. Poster presented at *ASHG 2020 Virtual Meeting*. Oct 27-30, 2020.
4. Labcorp internal data.