The Journal of Molecular Diagnostics, Vol. 16, No. 3, May 2014

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

Development and Performance of a Comprehensive Targeted Sequencing Assay for Pan-Ethnic Screening of Carrier Status

Alice K. Tanner,* C. Alexander Valencia,* Devin Rhodenizer,* Marina Espirages,* Cristina Da Silva,* Lisa Borsuk,† Sara Caldwell,† Edward Gregg,† Elizabeth Grimes,† Agnieszka M. Lichanska,† Leah Morris,† Anjan Purkayastha,† Brian Weslowski,† Clark Tibbetts,† Matthew C. Lorence,† and Madhuri Hegde*

From the Emory Genetics Laboratory, Department of Human Genetics, Emory University, Atlanta, Georgia; and TessArae LLC,† Potomac Falls, Virginia*

Accepted for publication December 11, 2013.

Address correspondence to Madhuri Hegde, Ph.D., Emory University Department of Human Genetics, 615 Michael St, Ste 301, Atlanta, GA 30322. E-mail: mhegde@emory.edu.

Identifying individuals as carriers of severe disease traits enables informed decision making about reproductive options. Although carrier screening has traditionally been based on ethnicity, the increasing ethnic admixture in the general population argues for the need for pan-ethnic carrier screening assays. Highly multiplexed mutation panels allow for rapid and efficient testing of hundreds of mutations concurrently. We report the development of the Pan-Ethnic Carrier Screening assay, a targeted sequencing assay for routine screening that simultaneously detects 461 common mutations in 91 different genes underlying severe, early-onset monogenic disorders. Mutation selection was aided by the use of an extensive mutation database from a clinical laboratory with expertise in newborn screening and lysosomal storage disease testing. The assay is based on the Affymetrix GeneChip microarray platform but generates genomic DNA sequence as the output. Analytical sensitivity and specificity, using genomic DNA from archived control cultures and from clinical specimens, was found to be >99% for all mutation types. This targeted sequencing assay has advantages over multiplex PCR and next-generation sequencing assays, including accuracy of mutation detection over a range of mutation types and ease of analysis and reporting of results. *(J Mol Diagn 2014, 16: 350–360; http://dx.doi.org/10.1016/j.jmoldx.2013.12.003)*

Carrier screening is genetic testing performed on asymptomatic adult individuals to determine their heterozygous status for mutations that can cause severe disease in their offspring. Identifying carrier individuals and couples provides them with a variety of reproductive options, including pre-implantation genetic diagnosis, use of donor gametes, prenatal testing, and adoption. Although carrier screening may be performed biochemically for some genetic disorders such as Tay-Sachs disease, for many disorders biochemical carrier testing is not available or carrier individuals may have inaccurate results, thus requiring molecular mutation analysis for accurate carrier detection.

Carrier screening for a specific inherited disease can be performed when there is a known family history of the disease and causative mutations have been identified. In addition, carrier screening can be performed on individuals without a family history on the basis of their ethnicity and genetic diseases that are known to occur with higher frequency in those ethnicities. For example, the American Congress of Obstetricians and Gynecologists (ACOG) recommends that all individuals of Ashkenazi Jewish (AJ) descent who are pregnant or considering pregnancy be offered carrier screening for founder mutations for four diseases known to occur at higher frequency in that population [Tay-Sachs disease, Canavan disease, cystic fibrosis

Supported by Emory Genetics Laboratory operating funds.

A.K.T. and C.A.V. contributed equally to this work.

Disclosures: L.B., S.C., E.Gre., E.Gri., A.M.L., L.M., A.P., B.W., C.T., and M.C.L. are employed by TessArae, LLC., which owns the GeneCipher algorithms, the PECS array, the reference and mutant synthetic templates, the PECS multiplexed primer mixes, and the AlleleConfirm Kit used in this study.

Copyright © 2014 American Society for Investigative Pathology and the Association for Molecular Pathology.
Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jmoldx.2013.12.003

(CF), and familial dysautonomia].[1] The recommendations of the American College of Medical Genetics and Genomics (ACMG) for carrier screening in individuals of AJ descent include the four diseases recommended by ACOG and also include founder mutations for five additional diseases (Gaucher disease, Niemann-Pick disease type A, mucolipidosis IV, Fanconi anemia group C, and Bloom syndrome).[2] Because other AJ founder mutations are identified in different diseases, these mutations and diseases are often added to AJ carrier screening panels offered by clinical testing laboratories. Other diseases for which carrier screening based on ethnicity is recommended include hemoglobinopathies and CF. Carrier screening for hemoglobinopathies is recommended for individuals of African, Southeast Asian, and Mediterranean descent.[3] Although carrier screening for CF was initially recommended for the non-Hispanic white population and those of AJ descent, it is now recommended that it be offered to all individuals, given the increase in individuals of mixed ethnicity and the difficulties that may be present in determining a person's ethnicity that is based on personal reporting.[4]

Given the changing ethnic admixture in the general population (the 2010 US Census indicated that more than half of the total US population growth between 2000 and 2010 was because of increases in the Hispanic population), the increasing use of donor gametes in assisted reproductive technology, and the decreasing cost of high throughput screening of many mutations simultaneously, it has been suggested that carrier screening be offered to the general population, regardless of ethnicity.[5–7] Another argument for pan-ethnic carrier screening is that newborn screening (NBS) programs, which include screening for CF and hemoglobinopathies, are not ethnicity based. Reasons for this include that general population-based screening is more equitable in avoiding missed diagnoses of treatable diseases[8] and the inaccuracy, incompleteness, and difficulty in obtaining ethnicity information,[8–10] both of which also apply to carrier screening. In addition, as more mutations and diseases are added to current ethnicity-based carrier screening panels, carrier frequencies for some disorders may approach those in the general population, such as in AJ panels that include screening for spinal muscular atrophy and fragile X syndrome.

The complexities of offering carrier screening for certain diseases only to specific ethnicities but offering carrier screening for other diseases to the general population could be alleviated by offering pan-ethnic carrier screening. The recent development of highly multiplexed carrier screening panels that are capable of concurrently reporting hundreds of causal mutations for many different monogenic diseases represents a far more informative and efficient approach than traditional sequential, single-gene testing. To this end, Emory Genetics Laboratory (EGL), in collaboration with TessArae, LLC, has developed a targeted sequencing assay for rapid routine screening that simultaneously detects 461 loci in 91 different disease-causing genes. This Pan-Ethnic Carrier Screen (PECS) assay focuses on disorders that are part of recommended NBS panels in addition to several other metabolic and lysosomal storage diseases. The disease-causing loci were chosen on the basis of prevalence in diverse ethnic populations and from EGL's proprietary mutation database that was amassed from the global patient populations referred to EGL for clinical testing. The PECS assay excludes some disorders, included in other commercially available carrier screening panels, that target milder and/or later onset diseases or rare mutations that are restricted to small populations. The assay is performed on the Affymetrix GeneChip microarray platform, a proven and standard laboratory technology for gene expression and cytogenetic analysis,[11] which uses a resequencing technology that generates gold standard genomic DNA sequence as the output,[12] unlike other panels developed on multiplexed PCR or hybridization probe platforms. Results of an extensive validation study that found the analytical sensitivity and analytical specificity of the assay, as well as results from a set of patient specimens, are presented in this report.

Materials and Methods

Microarray Design

The PECS assay is performed with an Affymetrix microarray-based targeted DNA sequencing platform. For each targeted mutation there are two detector tiles on the microarray. One detector tile is identical to the reference (wild-type; WT) allele sequence, and the other tile is identical to the mutant allele sequence. Each detector tile represents DNA sequences from the reference human genome sequence build 37 (hg19), and definitions of most of the targeted mutations are found in the Human Genome Mutation Database (BioBase). These detector tiles comprise overlapping sets of eight 25-base long oligonucleotide probes per base of sequence to be interrogated. The detector tiles enable reading of at least 24 bp, and, in a few cases, up to 101 bp of template DNA sequences in the immediate proximity of each targeted mutation locus. For a small number of loci representing large (>300 bp) deletions, the reference detector tile represents one of the two breakpoint sequences, whereas the mutant tile represents the junction sequence of the two breakpoints, and genomic sequences hybridizing to these tiles are amplified by two different primer pairs instead of the same primer pair as with the single nucleotide variations or small insertions and deletions.

Synthetic Template Design and Construction

To verify the presence and to assess the performance of all of the reference and mutant detector tiles on the array, 24 different synthetic DNA sequences that contain concatenated sets of approximately 20 different reference allele sequences each were manufactured by OriGene Technologies, Inc (Rockville, MD) and cloned into plasmids (pWT). A corresponding set of 24 different synthetic DNA sequences that contain concatenated sets of the mutant allele sequences were

Download English Version:

https://daneshyari.com/en/article/6112386

Download Persian Version:

https://daneshyari.com/article/6112386

Daneshyari.com