Cancer Genetics 224–225 (2018) 12–20

Cancer Genetics

ORIGINAL ARTICLE

Clinical germline diagnostic exome sequencing for hereditary cancer: Findings within novel candidate genes are prevalent

Zöe Powis[a,*], Carin R. Espenschied[a], Holly LaDuca[a], Kelly D. Hagman[b], Tripti Paudyal[c], Shuwei Li[d], Hiroto Inaba[e], Ann Mauer[f], Katherine L. Nathanson[g], James Knost[h], Elizabeth C. Chao[i], Sha Tang[b]

[a] *Ambry Genetics, Department of Emerging Genetic Medicine, CGC 15 Argonaut, Aliso Viejo, CA, 92656, USA;* [b] *Ambry Genetics, Department of Clinical Genomics, Aliso Viejo, CA, 92656, USA;* [c] *Ambry Genetics, Department of Genetic Specialists, Aliso Viejo, CA, 92656, USA;* [d] *Ambry Genetics, Department of Bioinformatics, Aliso Viejo, CA, 92656, USA;* [e] *St. Jude Children's Research Hospital, Department of Oncology, Memphis, TN, 38105, USA;* [f] *Creticos Cancer Center, Department of Medical Oncology, Chicago, IL, 60657, USA;* [g] *Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, 19104, USA;* [h] *Illinois Cancer Care – Peoria, Department of Medical Oncology, Peoria, IL, 61615, USA;* [i] *Division of Genetics and Metabolism, Department of Pediatrics, University of California, Irvine, CA, 92617, USA*

Abstract

Clinical diagnostic exome sequencing (DES) has been effective in diagnosing individuals with suspected genetic conditions; nevertheless little has been described regarding its clinical utility in individuals with a personal and family history of cancer. This study aimed to assess diagnostic yield and clinical characteristics of pediatric and adult patients undergoing germline DES for hereditary cancer. We retrospectively reviewed 2171 patients referred for DES; cases with a personal and/or family history of cancer were further studied. Of 39 cancer patients, relevant alterations were found in eight individuals (21%), including one (3%) positive pathogenic alteration within a characterized gene, two (5%) uncertain findings in characterized genes, and five (13%) alterations in novel candidate genes. Two of the 5 pediatric patients, undergoing testing, (40%) had findings in novel candidate genes, with the remainder being negative.

We include brief case studies to illustrate the variety of challenging issues related to these patients. Our observations demonstrate utility of family-based exome sequencing in patients for suspected hereditary cancer, including familial co-segregation analysis, and comprehensive medical review. DES may be particularly useful when traditional approaches do not result in a diagnosis or in families with unique phenotypes. This work also highlights the importance and complexity of analysis of uncharacterized genes in exome sequencing for hereditary cancer.

Keywords Diagnostic exome sequencing, Hereditary cancer, Germline testing, Novel genetic etiology.

Introduction

Since 2011, clinical diagnostic exome sequencing (DES) has been available as an effective tool for genetic diagnosis in patients with unexplained neurodevelopmental conditions and in cases in which the underlying etiology is believed to be genetic. Its utilization has allowed select patients who had

Received June 5, 2017; received in revised form March 12, 2018; accepted April 2, 2018

* Corresponding author.
E-mail address: zpowis@ambrygen.com

2210-7762/$ - see front matter
https://doi.org/10.1016/j.cancergen.2018.04.002

previously undergone a series of costly, invasive and often uninformative tests to receive a definitive diagnosis and end the diagnostic journey [1–5]. The diagnostic yield and utility of clinical DES has not been specifically studied in relation to patients suspicious for a diagnosis of hereditary cancer.

In recent years, multi-gene panel testing has increased our understanding of and ability to diagnose hereditary cancer, with positive rates ranging from 3.6–16.0%, depending on the cohort and type(s) of cancer studied [6–10]. Previous studies utilizing exome sequencing have analyzed the genetic findings in probands with specific types of cancers [11–13]. Two recent studies found germline mutations in cancer-predisposing genes in 8.5% and 10% of pediatric cancer patients, respectively; however, a causal relationship between the mutation and the patient's cancer was not established [14,15]. We aimed to characterize findings of a clinical laboratory cohort of unselected pediatric and adult patients referred for DES for suspected hereditary cancer conditions with personal and/or family cancer histories and assess the diagnostic yield of clinical DES for hereditary cancer indications. We report a rate of 21% potential relevant findings and include several brief case studies to illustrate the variety of issues that a clinician may encounter when pursuing DES.

Materials and methods

Terminology

Characterized disease gene: A gene known to underlie at least one Mendelian genetic condition.

Novel candidate gene: A gene that is not currently known to underlie a Mendelian genetic condition.

Clinical diagnostic exome sequencing: Whole-exome sequencing performed in the clinical setting in a Clinical Laboratory Improvement Amendments (CLIA) – certified diagnostic laboratory for single-patient diagnostic purposes.

Clinical validity: Based on the existing literature and knowledge about gene–disease relationships, clinical validity is the determination that a particular disease is truly caused by pathogenic variants in a particular gene.

Patient population

Patients with personal and family histories of cancer were ascertained sequentially from 2171 samples sent to Ambry Genetics Laboratory for clinical DES. Per our laboratory's standard DES protocol, clinicians were encouraged to refer all first-degree and other informative family members to aid in the proband's analysis and interpretation (trio testing and cosegregation studies). The patients' clinical and testing histories, along with pedigrees provided by referring physicians were carefully reviewed and summarized for each case by a team of genetic counselors. Patients with a personal and family history of cancer but with additional findings not traditionally associated with hereditary cancer syndromes (such as intellectual disability, dysmorphic features, hearing loss, and heart defects, etc.) as the major indications for testing were excluded from this analysis. Patients were included for this analysis if a suspected germline hereditary cancer predisposition due to personal and family history of cancer was identified as the main indication for testing by the provider. Patients were consented for testing by the ordering provider. Solutions Institutional Review Board determined the study to be exempt from the Office for Human Research Protections Regulations for the Protection of Human Subjects (45 CFR 46) under category 4.

DES and variant analysis

Patients' clinical and testing histories, along with pedigrees provided by referring physicians, were reviewed and summarized by a team of board certified genetic counselors with previous clinical experience. Genomic DNA was isolated from whole blood from all probands. Exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described [3,16]. All relevant alterations were confirmed by automated fluorescence dideoxy (aka "Sanger") sequencing.

Primary findings related to the patient's phenotype were reported. Interpretation was based on the clinical, family, and test information provided by the referring provider and the knowledge of genes and alterations at the time of reporting. Each gene was then assessed for the level of phenotypic overlap between the proband and reported patients. Significant clinical correlation with previously reported patients and consistent inheritance pattern and disease mechanism (gain versus loss of function) were required to classify alterations in characterized gene(s) as relevant findings.

The overall clinical DES results categories were classified as one of the following: positive/likely positive characterized gene finding, uncertain characterized gene finding, candidate novel gene finding, suspected novel candidate gene finding, or negative (Supplemental Fig. 1). Genes were classified as either uncharacterized or characterized Mendelian disease-causing genes based on Ambry's clinical validity assessment criteria [17]. Overall DES results were deemed positive/likely positive if a pathogenic/likely pathogenic mutation was identified in a gene with positive/ likely positive phenotypic overlap with the patient's phenotype (Supplemental Fig. 1). Structural modeling by PhD computational structural biologists and additional *in silico* tools may also have been utilized for the alteration classification.

Analysis of novel candidate genes followed an internally developed assessment scheme [16]. Briefly, this is a transparent, comprehensive, and standardized sorting criteria for the evaluation and clinical reporting of novel genetic etiologies. The criteria are based on the evaluation of overlap of the proband's phenotype and evidence including human microdeletion/duplication syndromes, *in vivo* animal models, gene function, expression, and protein family and pathway information. Classification of alterations in characterized genes followed Ambry's clinical variant classification scheme (http://www.ambrygen.com/variant-classification) which incorporates published recommendations and guidelines by the American College of Medical Genetics and Genomics (ACMG) [6,18]. Calculation of diagnostic rates in characterized disease genes was based on all probands, since characterized genes were analyzed for all probands. Calculation of novel gene detection rates was based on the number of probands in whom this analysis was performed.