P623

Cytogenetic findings in a clinical next generation sequencing panel for very early onset inflammatory bowel disease

Hadia Hijazi[1], Tamara Luke[1], Kieran Pechter[1], Archana Tare[1], Kajia Cao[1], Laura Conlin[2], Judith Kelsen[3], Nancy Spinner[2], Yavuz Bayram[2]

[1]Division of Genomic Diagnostics, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, PA; [2]Division of Genomic Diagnostics, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA, Department of Pathology and Laboratory Medicine, The Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [3]Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA, Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Introduction: Inflammatory bowel disease (IBD) is characterized by chronic and recurrent inflammation in the gastrointestinal tract caused by defects in the intestinal mucosal barrier function. Very early onset inflammatory bowel disease (VEO-IBD) is the predominant monogenic form of all IBDs and is defined as IBD with an age of onset below six years. VEO-IBD is a genetically heterogeneous disease; therefore, genetic testing of children with VEO-IBD is often performed via genome-wide (genome or exome) next generation sequencing (NGS) platforms in which all genes are sequenced but only a panel of selected relevant genes is analyzed. While not intended for whole exome or genome analyses, the use of genome-wide NGS in panel testing can prioritize the analysis of incidental findings, here defined as copy number variants (CNVs) or chromosomal abnormalities that do not involve genes in the VEO-IBD panel. Additionally, genome-wide NGS platforms enable the determination of boundaries of cytogenetic variants that extend beyond a single VEO-IBD panel gene.
Methods: Samples from 424 children presenting with VEO-IBD underwent exome sequencing (ES; 362/424 cases) or genome sequencing (GS; 62/424 cases), followed by targeted sequence and copy number analyses of 98 VEO-IBD panel genes. Quality control metrics including the alignment, coverage, and contamination data were evaluated prior to analysis of the data. Bioinformatic analysis was performed using an in-house developed pipeline. After alignment and variant calling, raw sequence variants were filtered to retain rare variants in the targeted VEO-IBD regions of interest (ROIs). CNVs were called using data from the entire ES/GS backbone after which non-ROI CNVs were filtered out. Retained CNVs were annotated by their gene content, functional consequences, and population allele frequency. Pathogenic, likely pathogenic and variants of uncertain significance in ROIs were reported. Additionally, an incidentally detected chromosomal abnormality that is pathogenic but does not encompass any of the ROIs, was reported and interpreted in light of the available medical history.
Results: Eight of the 424 samples tested were abnormal, with five having pathogenic or likely pathogenic sequence variants in five genes: *WAS, FOXP3, SKIV2L, CYBB*, and *RET*. Three cases had cytogenetic abnormalities including a (suspected mosaic) trisomy 8, an unbalanced translocation, der(5) t(5;11)(q35.3;q21), resulting in the duplication of 11q21 to qter and the deletion of 5q35.3 to qter, and a mosaic trisomy 9. Since our VEO-IBD panel contains genes in chromosomes 8 and 11, trisomy 8 and the unbalanced translocation involving chromosome 11q were identified via routine panel analysis. Trisomy 8 was confirmed via chromosomal SNP array and reported as likely diagnostic because of a known association between mosaic constitutional trisomy 8 and Behçet syndrome, an inflammatory disease that can present with gastrointestinal symptoms. The terminal duplication of chromosome 11q involves panel genes and was therefore detected via routine analysis. Review of the breakpoints of this duplication and genome-wide CNV data revealed that this duplication is part of an unbalanced translocation event involving terminal deletion of chromosome 5q which does not include any ROIs. Follow-up Sanger sequencing of the breakpoints confirmed the presence of derivative chromosome 5 in this patient. The third cytogenetic finding, mosaic trisomy 9, was incidentally identified and has risen to analysis by virtue of flagging the sample for potential contamination during quality check. Since our panel doesn't contain any gene in chromosome 9, this finding was reported as an incidental finding.
Conclusion: Although the overall diagnostic rate of our VEOI-BD panel is low (8/424, ~2%), this data demonstrates the utility of NGS genome backbone coverage in VEO-IBD panel testing. Extensive CNV analysis with a cytogenetic approach can maximize the diagnostic yield of gene panel testing and facilitate the identification of clinically unsuspected underlying diagnoses.

https://doi.org/10.1016/j.gimo.2024.101529

P624

Performance and impact of clinical genome sequencing in patients with suspected rare genetic diseases in Peru

Erin Thorpe[1], Milagros Duenas-Roque[2], Mario Cornejo-Olivas[3], Jeny Bazalar-Montoya[4], Nelson Purizaca-Rosillo[2], Richard Rodriguez[3], Karina Milla-Neyra[3], Carlos De La Torre-Hernandez[2], Elison Sarapura-Castro[5], Carolina Galarreta Aima[6], Gioconda Manassero-Morales[4], Giulliana Chávez-Pasco[4], Luis Celis-García[4], Jorge La Serna[3], Ryan Taft[1]

[1]Illumina; [2]Hospital Nacional Edgardo Rebagliati Martins, EsSalud, Lima, Peru; [3]Neurogenetics Research Center, Instituto Nacional de Ciencias Neurológicas, Lima, Peru; [4]Instituto Nacional de Salud del Niño San Borja, Lima, Peru; [5]Instituto Nacional de Ciencias Neurológicas, Lima, Peru; [6]National Institutes of Health

Introduction: Molecular genetic testing has limited access in many regions across the world, especially in developing countries. The iHope program philanthropically provides clinical genome sequencing (cGS) to individuals with signs or symptoms of rare genetic diseases and limited or no access to molecular genetic testing.
Methods: We describe the performance and impact of cGS in a cohort of 247 patients drawn from three clinical sites in Lima, Peru. All patients were evaluated by a clinical geneticist and had signs and symptoms suggestive of a rare genetic disorder and limited access to molecular testing. Additional clinician patient selection criteria included a broad differential diagnosis (222/247, 89.9%), no other available next-generation sequencing test (198/247, 80.2%), non-diagnostic prior testing (180/247, 72.9%), a diagnostic odyssey lasting more than two years (173/247, 69.6%), a phenotypic presentation that the clinician assessed as severe (169/247, 68.4%), clinical suspicion of a disorder with an available treatment (91/274, 36.8%), parents of reproductive age (85/247, 34.4%), acute illness or ICU admission (29/247, 11.7%), and/or a first-degree relative with a similar clinical presentation (41/247, 16.5%).

Results: The cohort was comprised mostly of children (198/247, 80.2%). Patients traveled an average of 195 km for evaluation (range 0- 1011 km), 36.8% (91/247) of which resided outside of the capital city of Lima. Mean time from symptom onset to cGS testing was 8.48 years (range 1.8 months-57.4 years). Although most patients had at least one genetic test prior to cGS (175/247, 70.9%), the most frequently ordered test was a karyotype (132/247, 53.4%) and a minority had a prior test other than a karyotype (85/247, 34.4%). The diagnostic yield of cGS was 54.3% (134/247), with candidate variants inclusive of variants of unknown significance reported in an additional 21.5% (53/247). Clinician review of uncertain results endorsed that 69.8% (37/53) were clinically suspected to be likely positive based on clinical correlation with the patient phenotype. Reported variants included SNVs (162), small indels (46), CNVs (30) ranging in size from 3kb to 77Mb and including two probands with mosaic aneuploidy and two probands with multiple de novo CNVs suggestive of a derivative chromosome in a parent, STRs (5), mitochondrial SNVs (3), regions of homozygosity suggestive of uniparental disomy (1) and biallelic absence of the SMN1 c.840C allele (1). cGS results impacted clinician diagnostic evaluation in 85% (210/247) of cases. Changes in management were reported in 71.3% (176/247), inclusive of referrals (64.7%, 160/247), therapeutics (26.3%, 65/247), laboratory or physiological testing (25.5%, 63/247), imaging (19%, 47/247), and palliative care (17.4%. 43/247). cGS test results impacted genetic counseling in 72.1% (178/247). A notable case example includes a 3-month-old female in the ICU on mechanical ventilation from one month of life with liver failure, collateral circulation ascites, thrombocytopenia, respiratory infections and sepsis with a poor prognosis. At three months of age, cGS detected a homozygous, pathogenic variant in the GALT gene consistent with galactosemia, a condition which is not included in Peru's limited newborn screening program. Her diet was changed to soy formula, resulting in regained consciousness, independent breathing, and resolved visceromegaly. Unfortunately, prolonged mechanical ventilation resulted in tracheomalacia requiring a tracheostomy. She is currently 2 years old and able to stand and babble.
Conclusion: These findings indicate that increased availability of cGS testing in Peru will enable more robust and systematic clinical genetic diagnostic evaluations and support improved patient management.

https://doi.org/10.1016/j.gimo.2024.101530

P625

Utility of highest pathogenic variant frequency approach for application of BA1/BS1 ACMG criteria to reduce variants of uncertain significance

Kyle Salsbery[1], Devin Oglesbee[1], Elizabeth Selner[1], Angela Pickart[1], Zhiyv Niu[1], Emily Lauer[1], Dawn Peck[1]

[1]*Laboratory Genetics and Genomics, Department Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN*

Introduction: Variants of Uncertain Significance (VUS) continue to be reported at high rates as the gene count on panels increases. Overreporting of variants with unclear clinical significance can be frustrating for patients and providers. There is a need for laboratories to improve classification processes to reduce the number of unnecessary VUS reported. Mayo Clinic Laboratories (MCL) has a total of 8,733 submissions to ClinVar with VUS classifications accounting for 57% of those submissions. Allele frequency thresholds are used to determine if the minor allele frequency of a detected variant is greater than expected for an associated disorder, which can provide evidence that a variant is benign. These cut-offs can be calculated multiple ways, such as using phenotypic prevalence data or allele frequency calculators. These methods can be limited by a lack of accurate population data for rare conditions and newly described genes. Prevalence data can also vary widely in published literature and between populations. Allele frequency calculators require multiple variables, including allelic heterogeneity, genetic heterogeneity, and penetrance, which are often unknown or not well described for many conditions included on large panel tests. Here, we describe an additional approach of utilizing the highest allele frequency of the most common pathogenic/likely pathogenic variant reported in a gene to curate frequency thresholds for applying BS1/BA1 ACMG criteria and to decrease the number of reported VUS.
Methods: An external public database was utilized to identify the highest sub-population allele frequency among all pathogenic/likely pathogenic variants reported in a specific gene from the gnomAD population database. The allele frequency of this variant is multiplied by a factor of 1.5 or 10 to create a gene specific cut-off in applying BS1 or BA1 ACMG criteria respectively. Prior ClinVar entries from inborn errors of metabolism and neurology panel tests with a VUS classification and conflicting ClinVar submissions towards a benign classification were retrospectively reviewed to determine the impact on classification utilizing these new cut-offs.
Results: A total of 50 variants previously classified as VUS in ClinVar were reviewed. These variants were reported in 32 different genes from inborn errors of metabolism and neurology gene panels. In 28% (14/50) of these variants, BS1 criteria was applied utilizing the new cut-offs based on the highest pathogenic variant frequency approach. In 4% (2/50) of these variants, BA1 criteria could be applied utilizing the new cut-offs. In total, 32% (16/50) of variants had BS1/BA1 criteria applied that was not originally applied at the time of the initial variant evaluation. In 22% (11/50) of variants reviewed, the previous VUS classification would be downgraded to likely benign or benign utilizing the new cut-offs. In the remaining 5 variants that were not reclassified with the added criteria, BS1 was the only criteria applied so the variant would remain a VUS based on current ACMG classification guidelines.
Conclusion: Utilizing the highest pathogenic variant allele frequency reported in a gene to aid in applying ACMG criteria resulted in a 22% reduction of VUS and increased application of BS1/BA1 criteria. It is notable that multiple approaches may be needed to curate allele frequency thresholds in classifying molecular variants and each calculation has its own benefits and limitations. Limitations to this specific method include consensus on which variant is the most common pathogenic/likely pathogenic variant in the gene of interest, newly discovered genes with few reported pathogenic variants, how to account for pathogenic variants with outlying frequencies, and incomplete reference populations. We anticipate that applying a highest pathogenic variant allele frequency cut-off will continue to decrease the number of reported VUS on gene panel testing in our laboratory.

https://doi.org/10.1016/j.gimo.2024.101531