Genetics

G01. Validation of the Ion S5 and Ion Chef for Cystic Fibrosis Mutation Analysis

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Introduction: Cystic Fibrosis (CF) is a common autosomal recessive genetic disorder that is caused by mutations in the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene. There have been more than 1900 different mutations found within the *CFTR* gene. Current commercially available *CFTR* mutation panels are limited in the number of clinically significant mutations that can be detected. In this study, performance of *CFTR* mutation analysis from whole blood using the Ion S5 next-generation sequencing (NGS) and the Ion Chef was evaluated. **Methods:** Samples (n=45) were tested utilizing a combination of retrospective whole blood samples previously genotyped using the xTAG Cystic Fibrosis (CFTR) 39 kit v2 (n=35) and DNA from previously characterized Coriell Institute samples (n=10). DNA from these samples was extracted using Roche MagNA Pure 96 system with the DNA Isolation Kit DNA/Viral NA SV 2.0. All DNA samples were quantified using the Qubit dsDNA HS assay on the Qubit 3.0 Fluorometer. Ten nanograms of DNA from each sample was used to prepare pooled barcoded libraries on the Ion Chef using Ion AmpliSeq Kit for Chef DL8 with the Ion Ampliseq CFTR Panel. Pooled libraries were combined and diluted to 50 pM and templated on the Ion Chef with the Ion 520 & Ion 530 Kit-Chef and loaded onto an Ion 520 chip. The Ion 520 chip was sequenced on the Ion S5. Torrent Suite software was used to analyze the data and compare to the *CFTR* human genomic sequence as a reference (hg19). A filter chain was applied to the data to analyze the *CFTR* gene for 97 mutations known to cause CF. **Results:** Analysis NGS data from 45 specimens, revealed *CFTR* gene analysis by NGS has excellent analytical sensitivity and analytical specificity [100% positive predictive value (PPV)/ 100% negative predictive value (NPV)]. The sample set included 25 patients with previously identified mutations in the *CFTR* gene and 20 patients with wild-type *CFTR* genes. Inter-assay variability was assessed by running the same 3 specimens on 2 independent runs. Intra-assay variability was determined by analyzing 3 specimens in duplicate within the same run. Analysis of the NGS data produced the expected result for all samples. **Conclusions:** These results demonstrate the benefit of *CFTR* mutation analysis from whole blood using the Ion S5 and the Ion Chef. Total technologist hands-on time is 3 hours for 24 samples. The ease of the Ion Chef workflow allows NGS to become feasible for a mid-sized clinical laboratory. Use of this NGS assay will ensure efficient, reliable and reproducible analysis of the *CFTR* gene.

G02. Reinterpreting Previously Reported Genetic Variants is Clinically Significant

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Introduction: Next-generation sequencing (NGS) testing has become a common diagnostic tool for evaluating pediatric patients with intractable epilepsy. NGS testing can comprehensively identify gene variants of interest; however, determining the clinical significance of novel variants is challenging. Advances in publically available databases and standardized interpretation criteria have improved the clinical interpretation of variants. However, the utility of re-interpreting previously reported NGS epilepsy tests has not been systematically evaluated. In this study we identify the frequency and significance of variant reclassification from previously reported NGS epilepsy gene panels. **Methods:** All epilepsy NGS epilepsy gene panel reports from patients at a tertiary-care pediatric hospital were retrospectively reviewed (July 2012 to August 2015). Reports were from one reference laboratory (GeneDx), which only reported variants as pathogenic/likely pathogenic (P-LP), or uncertain in significance (VUS). Previously reported variants were re-evaluated using population (ExAC, 1000 Genomes phase 3) and clinical (HGMD, ClinVar) databases. Any variant with a high population frequency (>1%) or a new report in a clinical database was reinterpreted using ACMG variant classification criteria. **Results:** Three-hundred and 7 patients were previously tested yielding 308 variants in 184 patients. Of the 308 variants, 19% were P-LP (n=59), and 81% were VUS (n=249). 19% (n=11) of the P-LP variants were reclassified; variants were downgraded to VUS (n=4), or B-LB (n=6), and one was upgraded to pathogenic (*KCNQ2*) Of the 249 VUS reported, 14 did not have sufficient variant information (transcript, genomic coordinate, other) to be reinterpreted. Of the remaining 235 VUS, 31% (n=72) were reclassified. Variants were downgraded to B-LB (n=65) or upgraded to P-LP (n=7, *MECP2*, *TBC1D24*, *TSC1*, *PRICKLE1*, and *SCN8A*). Overall, 66 of 307 patients (21.5%) were impacted by variant reclassification. Reclassification rate decreased from tests initially reported in 2012 (37%) to 2015 (25%) (m= -4%/yr, R^2=0.99, p=0.007). **Conclusions:** Based on our single institution experience, a large proportion of variant classifications were revised (26.9%; 83/308). These reclassifications impacted 21% of patients (66 /307). A 3% gain in diagnostic yield was obtained by reinterpreting previously reported VUS. In addition, many reclassified VUS were downgraded in clinical significance. Overall, these findings indicate that all P-LP and VUS variants from previously reported NGS tests should be routinely reviewed and reinterpreted.

G03. Hypertrophic Epicardial Adipose Tissue is a Source of EPAC Proteins Directly Associated to ST2 Production and Heart Dilation and may be a Potential Index of Heart Remodeling in CVDs Patients

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Introduction: Epicardial adipose tissue (EAT) is a myocardial fat from which released molecules can directly reach the heart. In pathological conditions, the ubiquitously tissue hypertrophy mediators are the exchange proteins directly activated by cAMP (EPACs), named EPAC1 and EPAC2. In the heart the main protective signalling against detrimental remodelling is the ST2 and IL33 molecules. ST2 exists both as transmembrane receptor (ST2L) and soluble (sST2) form and in case of physiological stretch ST2L bind IL33 promoting anti-fibrotic signals. Contrarily in CVDs, sST2 is up regulated functioning as scavenger of IL33 and promoting heart dilatation. Interesting is that ST2 can be also produced by adipose tissue in normal condition. Due to EPACs properties to induce hyperplasia, our hypothesis is that larger EAT cells may also produce sST2 that can local amplify its detrimental role on myocardium. For these reasons we wanted to verify in CVDs patients first if larger EAT cells are able to up-regulate EPAC proteins and second if EPACs may be associate to sST2 EAT production and heart dilatation. **Methods**: Fifty CVD patients are enrolled and stratified according to EAT median thickness (8mm). Plasma and EAT biopsies were collected during surgery. Indexed left ventricular mass (hLVM), end-diastolic posterior wall (EDPW), relative wall thickness (RWT), left ventricular mass (LVM) values were used as cardiac dilatation indexes commonly approved in clinical practice. Gene expression and protein assays were performed to investigate EPACs, ST2, IL33 mRNA and protein production. **Results**: Our data demonstrated that CVDs patients with EAT >8mm have significantly positive correlation with RWT and they also presented higher EPAC1 and ST2 mRNA and protein levels than CVDs patients <8mm; contrarily IL33 protein is down regulated in CVDs >8mm and up-regulated in CVDs patients < 8mm. CVDs patients with hypertrophic EAT both EPAC1 and EPAC2presented positive correlation with hLVM, EDPW, LVM indexes and ST2 mRNA levels. **Conclusions**: Our results demonstrated in EAT, EPACs are directly associated to EAT hyperplasia and sST2 local production suggesting their implication in detrimental heart hyperplasia due to the positive correlation between EPAC1 and EPAC2 versus cardiac indexes and ST2. From these results we can suggest that EAT thickness can be a potential newer parameter of detrimental heart remodelling in the prevention of CVDs complications.

G04. Discovery of a Novel, Accurate Tagging SNP for HLA-B*15:02 Screening Before Carbamazepine Therapy in the Multiethnic United States Population

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Introduction: The gene *HLA-B*15:02* is strongly associated with life-threatening severe skin hypersensitivity reactions such as Stevens-Johnson Syndrome and toxic epidermal necrolysis in patients treated with carbamazepine (CBZ) and structurally-related medications. FDA-approved labeling information for CBZ recommends screening for *HLA-B*15:02* prior to CBZ therapy in patients of Asian ancestry due to its high frequency in Asian populations. In this study, we aimed to a) identify a direct and accurate method for the detection of *HLA-B*15:02* as a screening tool, and b) evaluate the prevalence of *HLA-B*15:02* in a large cohort of US patients. ***Methods:*** *HLA-B* references deposited with the World Health Organization Nomenclature Committee for Factors of the HLA Systems were compared to identify a candidate tagging single-nucleotide polymorphism (SNP) for *HLA-B*15:02.* Data from public research databases as well as from approximately 30,000 patient samples were used to test concordance of *HLA-B*15:02* with the candidate tagging SNP. Ethnicity information of patients with positive *HLA-B*15:02* results were evaluated with respect to current information about ethnicity and *HLA-B*15:02* carriership. ***Results:*** We identified a SNP in *HLA-B* as a candidate tagging SNP for *HLA-B*15:02.* Samples from the 1,000 Genomes Project that were typed for *HLA* (n = 955) and sequenced fully showed 100% concordance between *HLA-B*15:02* and the minor allele of the tagging SNP. Furthermore, in a large study cohort of US individuals (n = 29,194), sequencing data demonstrated that the minor allele was a reliable marker for *HLA-B*15:02* detection with 100% sensitivity and 99.97% specificity (P = 0.0001 by two-tailed Fisher's exact-test). Notably, out of 158 positive individuals detected in this study, 65 had physician-reported ethnicity information, including 28 (43%) Asians, 14 African Americans (22%), 11 Caucasians (17%), 2 Hispanics (3%), and 10 'Other' ethnicity (15%). ***Conclusions:*** We have discovered a novel tagging SNP within the *HLA-B* gene that is useful as a direct and accurate method for *HLA-B*15:02* screening. The SNP is strongly linked with *HLA-B*15:02,* with all true positive carriers in this study also showing carriership. Our data demonstrates that it is a good screening tool to identify potential *HLA-B*15:02* carriers in the US population. Finally, we show that screening patients for *HLA-B*15:02* based on observed or self-reported Asian ancestry may be insufficient to identify a large portion of potential carriers of this allele in the ethnically diverse US population.

G05. Spectrum of Mutations in *Hbb* Gene Among Thalassemia Major Patients in a Cohort of Nepalese Population

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Introduction: The thalassemias are the most common monogenic disorders with a genetically determined reduction in the rate of one or more types of normal haemoglobin polypeptide chain resulting in a decrease in the amount of haemoglobin involving the affected chain. Beta thalassemia is a highly heterogeneous disorder in its phenotype, geographical distribution and molecular mechanism. **Methods:** DNA was extracted from the 26 clinically diagnosed blood sample and Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS PCR) was used for amplification to analyze mutations in the *hbb* gene and 2% gel electrophoresis was used for visualization of PCR products. **Results:** Among 26 β-thalassemia major patients, 13 (50%) had IVS 1-5 (G>C) mutation, 8 (30.76%) had 619bp deletion, 2 (7.69%) CD 8/9 (+G), 1 (3.84%) CD 15 (G>A), 1 (3.84%) had -88 (C>T) mutation whereas CD41/42 (-TCTT) was not detected in any of the patients. Among the patients with 619 bp deletions 2 (25%) were homozygotes and 6 (75%) were heterozygotes. **Conclusions:** This is the baseline study to assist in the regulation of proper new health policies which will impact in the proper diagnosis and treatment.

G06. Custom NGS Panels from Optimized Gene Sets for Inherited Disease Research

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Introduction: AmpliSeq is a next generation sequencing library preparation method for targeted resequencing that utilizes highly multiplexed PCR to amplify regions of interest. A key to successful AmpliSeq libraries is the primer panel used for target amplification. Until now primers have been available as ready-to-use panels or as custom made-to-order panels. We describe a new process for creating customized panels from optimized PCR primers called Ion AmpliSeq On-Demand panels. Primers for On-Demand panels are available as whole gene sets (all of the primers needed to create libraries that cover the entire coding regions of genes) and are selectable on the AmpliSeq.com website by either uploading gene lists or choosing genes from the content selection engine base on disease research areas. Predicted gene coverage metrics are also available on the website as part of the content selection process. **Methods:** Ten disease research On-Demand panels were designed on AmpliSeq.com using the content selection engine. Libraries were prepared from a variety of sample types (purified reference genomes, fresh, frozen, and dried blood, cheek swabs, and saliva) by both manual and automated methods and sequenced on several different Ion Torrent sequencing systems. Sequencing data were analyzed for overall panel uniformity (% bases in target ≥ 0.2X mean coverage), on target (% of all HG19-mapped reads that also map to designed targets), reproducibility (CVs of panel uniformity across library preparation methods and sample types), and specificity and positive predictive value of variant calling for specific reference genomes tested. **Results:** Coverage uniformity for all On-Demand panels was ≥ 95% across all library preparation methods and sample types. Sensitivity and positive predictive value of variant calling for reference genomes for single nucleotide polymorphisms were ≥ 97%. Fifty-nine genes recommended by the American College of Medical Genetics and Genomics for reporting of incidental findings (ACMG59) were tested as a stand-alone panel and together with 75-135 additional genes and their uniformity was shown to be ≥ 97% in all contexts. **Conclusions:** Ion AmpliSeq On-Demand panels provide a powerful and flexible new tool for human inherited disease research. Primer designs for genes of interest can be mixed and matched to fit specific research interests while maintaining high performance in sequencing applications. Inventory is expanding to ~5000 genes in the near future, covering most important genes with links to inherited diseases.

G07. WITHDRAWN

G08. Comprehensive Carrier Testing of 9,785 Chinese Couples for Common Severe Recessive Disorders

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Introduction: China, as a populous country, is characterized by multi-ethnicity, complicated substructure, widely geographical distribution, as well as cultural diversity, with long history of consanguineous marriage, leading to high carrier rates of recessive diseases or detrimental mutations in some regions of China. With development of society, the population mobility is strengthened, making some highly prevalent genetic diseases in specific regions become high incidence and carrier rate in the whole nation of China. However, a comprehensive expanded carrier screening for Chinese population had not been performed so far. Here, a comprehensive carrier testing based next generation sequencing was implemented for 8 prevalent and severe recessive disorders in Chinese population. **Methods**: A high-throughput method based targeted genes capture and NGS was employed to detect variants related 8 common disorders (Dystrophinopathies, Hemophilia, hereditary hearing loss, Galactosemia, Phenylketonuria, Wilson disease, Glycogen storage disease type II and Autosomal recessive polycystic kidney disease). The variants were interpreted by referencing the standards and guidelines recommended by ACMG. YH genome DNA and 179 samples with known variants characterized were analyzed to evaluate mutation detection power. Then carrier testing was performed in 9,785 couples from southern China after an informed consent process. **Results**: The results of validation samples indicted the analytical accuracy and sensitivity were all over 99%. Among 9785 couples, we analyzed all exons and selected intergenic and intronic regions of 13 target genes. 1612 (8.24%) individuals were found carrying 352 distinct pathogenic variants. Of these distinct pathogenic variants, 67 (19.03%) loss-of-function mutations were never been reported. 16 couples and 33 females were determined to be at high risk of having offspring with related autosomal recessive and X-linked disorders, respectively, signifying that approximately 1.25% of genetically affected births could be averted combined with genetic counseling and prenatal diagnosis after the performance of carrier testing. **Conclusions**: Combined with prenatal diagnosis, comprehensive carrier screening based NGS can generate great significance for averting genetically affected births for severe genetic disorders in multi-ethnicity and complicated substructure Chinese population.

G09. Exome Re-Analysis and Complementary Testing Identify Novel Mutations for Rare Mendelian Disorders

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Introduction: The advent of next-generation sequencing technologies, especially the introduction of whole exome sequencing has provided an opportunity to screen a patient's entire exome to establish genetic diagnosis. The utility of WES to identify novel genes and variants causative of Mendelian disorders has been clearly demonstrated in recent years. Most excitingly, there are at least a few dozen new genes/mutations are being discovered each year approved to be associated with these disorders. **Methods:** We re-analyzed patients' WES data with initial negative results for new clinical phenotype and/or in novel genes that have been recently discovered to cause Mendelian diseases. A new and lab developed data analysis pipeline Cincinnati Clinical Exome Pipeline Analysis Suite "CCEPAS" is utilized for the re-analyses. In addition, knowing the limitation of the WES, we were able to utilize a few complementary testing strategies, such as, deletion-duplication analysis, epigenetic testing and Sanger rescue sequencing to identify mutations that helped to reach a definitive diagnosis in patients that were tested to be "negative" or "inclusive". **Results**: We were able to establish new diagnoses in a good number of patients with rare complex Mendelian diseases. We have completed re-analyses in 19 cases. We were able to identify new findings in 4 families (21%) based on newly discoveries of gene function and/or disease correlation. For example, we have a patient with a suspected clinical diagnosis of CVID, but the initial WES yielded no positive findings. Two years later, the physician requested analysis for CECR1 gene, which was recently report in patients with recurrent infections and antibody deficiency without vasculitis, the latter being a key feature in patients with CECR1 mutations (leading to ADA2 deficiency). Re-analysis in this patient resulted in the finding of a nonsense mutation. Additional rescue sequencing revealed another non-coding likely pathogenic variant. Enzymatic activity of ADA2 was nearly absent in the patient's serum. **Conclusions:** Periodic re-analyses of WES data and complementary testing helped to establish definitive diagnosis in patients with rare Mendelian disorders.

G10. Validation of A Cystic Fibrosis 55 Mutation Screening Assay on the QuantStudio 12K Flex Open Array System

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Introduction: The QuantStudio 12K Flex Real-Time polymerase chain reaction (qPCR) system with Open Array block (QuantStudio; ThermoFisher Scientific, Waltham, MA) facilitates qPCR–based applications on a nanofluidic plate, running up to 3,072 reactions in parallel per array. In this study, an interdisciplinary review was conducted on the State of California and Kaiser Permanente Southern California (KPSC) patient population to determine a customized mutation panel that included the significant variants for cystic fibrosis (CF) mutation screening. The performance characteristics of the assay were validated. **Methods:** Mutation analysis review and determination of the customized CF variants to be tested was conducted by multiple clinical disciplines based on data from the State of California Newborn CF Screening and the patient population characteristics of KPSC. Fifty-five variants were selected. De-identified clinical samples were used for the validation. DNA was extracted from EDTA whole blood samples using the QIAgen MDx and QIAsymphony instruments (QIAGEN, Hilden, Germany) and tested in 59 independent reactions (33nL each), run in duplicate, on the QuantStudio. Results were analyzed with the TaqMan Genotyper Software for cluster analysis and on the Quant Studio 12K Flex expression suite for amplification analysis. **Results:** After testing 338 de-identified patient samples and 54 assayed samples from Coriell and CAP, the accuracy of testing generated 100% concordant results compared to the reference method for 227 negative (wild type) and 165 positive (heterozygous or homozygous mutant) samples. Analytical specificity for all reactions was 99.76% and analytical sensitivity was 100% with a minimum requirement of 10 ng/µl of DNA. The inter- and intra- run precision studies generated acceptable results with 100%