GENETICS

G01. Detection of HLA-A*3101 by Real-Time PCR Assay

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Introduction: Carbamazepine is one of the most commonly prescribed drugs for the treatment of epilepsy, as well as trigeminal neuralgia and bipolar disorder. A minority of treated persons have hypersensitivity reactions that vary in prevalence and severity. The HLA-B*1502 allele has been shown to be strongly correlated with carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS–TEN) in the Han Chinese and other Asian populations. The presence of the HLA-A*3101 allele was associated with carbamazepine-induced hypersensitivity reactions among subjects of Northern European ancestry. The presence of the allele increased the risk from 5.0% to 26.0%, whereas its absence reduced the risk from 5.0% to 3.8%. We developed a real-time SYBR green melting curve assay that specifically detects HLA-A*3101. **Methods:** The real-time detection system consists of primers for amplifying human growth hormone (HGH) as control gene and the HLA-A*3101 allele. As the DNA amplifies, SYBR green intercalates into the double stranded DNA structure and the fluorescence increases greatly. During a dissociation step, the amplicon is incrementally increased in temperature until it reaches its melting temperature where the double-stranded DNA dissociates causing the fluorescence to decrease. The fluorescence is observed only if the primers bind and create the product thereby determining if the allele is present or absent. The control primer amplifies and gives a fluorescent signal for each sample to ensure DNA quality. To increase the specificity of the sequence specific primers, locked nucleic acid (LNA) primers were used. **Results:** We tested a total of 75 samples consisting of Coriell DNA and patient DNA. The sample set included HLA-A*2902, HLA-A*30.02, HLA-A*33.03 and HLA-A*2402 that are closely related alleles to HLA-A*3101. The primers were able to distinguish the closely related alleles from HLA-A*3101. Both the melting curves and Ct values are taken into account in making the allele calls. We found 5 HLA-A*3101 positive samples among the 75 samples tested mostly of European descent. To confirm the results, the HLA-A gene was amplified and exon 2 region sequenced; the sequencing results were concordant with the SYBR green real-time assay. **Conclusions:** Detection of HLA-A*3101 helps in predicting likelihood of hypersensitivity reactions to Carbamazepine and assisting physicians in customizing carbamazepine therapy. We developed a simple, rapid, and cost effective method for detecting HLA-A*3101. In real-time SYBR green melting curve assay the analysis and detection is done simultaneously that eliminates post PCR handling. This method has better specificity than serological methods and cost effective compared to sequencing.

G02. Development and Validation of a Diagnostic Test for Five Distinct Genetic Disorders with Heterogeneous Genetic Etiology Using Next-Generation Sequencing

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Introduction: Genetic testing of clinically heterogeneous group of disorders with a complex genetic etiology using Sanger sequencing can pose major challenges for molecular diagnosis. Next-generation sequencing (NGS) allows rapid and cost-effective detection of mutations in genes associated with multigenic disorders. In contrast to whole exome sequencing, where coverage is not complete, targeted NGS sequencing panels allow for comprehensive high quality coverage of genes of interest. **Methods:** For this study, five distinct genetic disorders were selected based on send out reports & client feedback. The CHOP_5D panel was designed to resequence the coding exons of 154 genes associated with five disorders (early infantile epileptic encephalopathy, craniosynostosis, noonan spectrum of disorders, hearing loss and paraganglioma-pheochromocytoma syndrome). The capture library (SureSelect) was optimized by rebalancing baits to improve coverage uniformity after examining the initial design. Enriched libraries were sequenced on the MiSeq. Sequence data were analyzed using a custom bioinformatics pipeline. Validation was performed on two HapMap samples and 33 previously characterized blinded clinical samples, of which 15 specimens were patients with disease causing mutations. Exons with coverage below 30x were Sanger sequenced for high analytical sensitivity. Data quality was evaluated based on average coverage, base quality scores and percentage of bases with 30x coverage. Inter-run and intra-run reproducibility was assessed using a standard set of alignment and variant metrics. Analytical sensitivity and specificity were evaluated by comparing genotyping results (obtained by Sanger sequencing or SNP array) to NGS results. **Results:** Re-balancing capture baits improved coverage uniformity dramatically. Percentage of exons with minimum per-base coverage below 30x dropped from 14.4% (>105 exons) to 4.6% (<40 exons) after bait redesign. On average, greater than 99% of bases within the region of interest had at least 30x coverage (mean coverage:600x). Consistent alignment and variant calling metrics were obtained with multiple triplicates demonstrating high reproducibility of this assay. Validation testing correctly identified all point mutations and indels in the 15 positive control samples; no false positives were identified in the negative control samples. The results demonstrate that both the analytical sensitivity and specificity of the assay are >99%.
Conclusions: We have validated a comprehensive NGS assay that, in contrast to whole-exome sequencing, will offer complete coverage and cost effective analysis of 154 genes associated with five distinct genetic disorders. This panel can provide treatment options and diagnostic advantage in cases with atypical clinical presentation and in cases where previous analyses have failed to identify a genetic defect.

G03. Mosaic DNA Methylation in Atypical Angelman Syndrome: A Case Report and the Development of a Molecular Technique to Improve Detection of Mosaic DNA Methylation in Prader-Willi and Angelman Syndromes

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Introduction: Here we report a 3-year-old boy conceived by *in vitro* fertilization (IVF) who presented with global developmental delay, absent speech, and an unsteady gait in the absence of other cardinal features but suspicious of Angelman syndrome (AS). Multiple independent Methylation Specific PCR (MS-PCR) tests using the patient's peripheral blood and buccal swab genomic DNA showed a normal paternal, but faint band for maternal *SRNPN* gene allele. Additional tests employing Methylation-specific Multiple Ligation Probe Assay and pyrosequencing showed complete absence of maternal allele, a result consistent with classical AS. The chromosome 15 UPD studies indicated biparental inheritance of chromosome 15. Imprinting Center deletion analysis did not reveal a deletion of the imprinting center region. We have developed and validated a sensitive semi-quantitative MS-PCR to improve the detection of mosaicism and/or incomplete imprinting defects of *SRNPN* gene, and to obtain a meaningful genotype-phenotype correlation on atypical/mild AS or Prader-Willi Syndrome (PWS) cases. **Methods:** This assay uses MS-PCR to determine the maternal or paternal origin of SNRPN gene within the PWS/AS critical region. Genomic DNA is treated with sodium bisulfate to convert unmethylated cytosines to uracil. To quantify the PCR band intensity, a multiplex PCR amplification reaction using fluorescently (FAM) tagged primers that amplify either the uracil-containing *SRNPN* locus (unmethylated, paternal DNA producing a 101bp amplification product) or the methylated cytosine-containing *SRNPN* locus (methylated maternal DNA producing a 177bp product). Amplification products are analyzed on an ABI 3130xl system for the presence of maternal and paternal *SNRPN* alleles. By analyzing and quantifying the amplicon band intensities on normal and mixed samples, a ratio was established to determine normal and mosaicism. Test validation studies showed reliable detection of 5% to 40% of somatic mosiacisim with this methodology.
Results: Here, we report the development and validation of a simple semi-quantitative, Fluorescent-MS-PCR-capillary gel electrophoresis based assay to reliably detect somatic mosaic and/or incomplete imprinting defect in a patient with exceptionally mild AS. **Conclusions:** Reliable detection of somatic mosaicism for imprinting defects both helped to explain the exceptionally mild clinical AS features observed in our patient, and resulted in better patient management and genetic counseling. This case highlight the inability of widely used tests employing regular MS-PCR, MS-MLPA and pyrosequencing to detect somatic/methylation mosaics for the *SNRPN* gene alleles and risk for molecular misdiagnosis. In addition, this case revealed another example of increased risk of imprinting defects observed in children born with assisted reproductive technology.

G04. The Creation and Implementation of a Clinical Exome and Exome Confirmation Pipeline

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Introduction: Whole exome sequencing (WES) has rapidly moved from the research laboratory to the clinical laboratory and is increasing in both use and utility for clinical testing. The benefits of WES remains the same for clinical diagnostics as

it does for the research setting, specifically sequencing large numbers of genes in a short time frame allowing for rapid evaluation of homogenous, ambiguous, or mild disorders and phenotypes. However, issues such as missing coverage and the high rate of false positives and false negatives associated with WES are amplified in the clinical setting. WES sequencing routinely identifies over 10,000 variants and typically 15% to 20% of the targeted exome is not covered well, with known disease causing genes at a higher rate of 30% to 40%. Exons with no or low coverage could be harboring a causative mutation and clinical labs must be able to verify that the mutations reported are a true positive. Many methodologies have been implemented including using simultaneous WES of parental samples and family members, but the Sanger sequencing is still the gold standard. **Methods:** To facilitate better detection of variants in the 5000 genes associated with disease in the Human Gene Mutation Database (HGMD), we have created a Clinical Exome that includes higher coverage of these genes and created an Exome Confirmation pipeline to rapidly confirm chosen variants through Sanger Sequencing. The addition of probes in the genes of interest allows for greater interrogation of disease related genes and the Sanger pipeline allows for efficient confirmation of variants. The Exome Confirmation pipeline combines PrimerFinder, an automated primer design script; GLOBE, an internally developed LIMS system; robotics; and Bioneer Pyrostart lypholized Master Mix plates to rapidly and reliably amplify and sequence variants selected for confirmations. Here we show the comparison data between the Clinical Exome and commercially available exomes as well as the detection and failure rates of the Exome Confirmation pipeline developed and currently in use at Emory Genetics Laboratory.
Results: The average coverage of HGMD listed genes was decreased from 36% to 11% in the Clinical Exome compared to commercially available products. The Exome Confirmation Pipeline boasts a 4% failure rate on the first PCR pass, with only 1% of the total needing manual redesign. **Conclusions:** The combination of the Clinical Exome and the Exome Confirmation pipeline allows for increased diagnostic utility of the test along with rapid validation of selected variants.

G05. SNP Microarray Analysis Reveals Whole Gene Deletion of the Mineralocorticoid Receptor in a Patient with Features of Pseudohypoaldosteronism Type 1

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Introduction: Pseudohypoaldosteronism type 1 (PHA1) is a rare disorder of mineralocorticoid resistance presenting in infancy with dehydration, hyponatremia, hyperkalemia, and failure to thrive. Renal unresponsiveness to aldosterone leads to salt-wasting despite increased plasma renin and aldosterone concentrations. Autosomal dominant PHA1 is caused by inactivating mutations of the mineralocorticoid receptor (MR) gene, *NR3C2*, located on chromosome 4q31.23. Many PHA1 patients with sporadic or inherited mutations of *NR3C2* have been reported, but gross deletions that include *NR3C2* have yet to be documented as a cause of PHA1. We report a large heterozygous deletion on chromosome 4, including the *NR3C2* gene, in a male who presented with hyponatremia (Na+ 125 mmol/l) and hyperkalemia (K+ >10 mmol/l) at 10 days of life. Normal 17-hydroxyprogesterone (26 ng/ml) excluded classic congenital adrenal hyperplasia. A mineralocorticoid pathway defect was suspected, and confirmed subsequently by determination of increased aldosterone (3,475 ng/dl) and plasma renin activity (PRA, 560 mg/ml/hr), after the first aldosterone measured low (2 ng/ml). Renal ultrasound showed mild left pelvocaliectasis without hydroureter. SNP microarray analysis was obtained because the patient also had partial anomalous pulmonary venous return. **Methods:** SNP microarray analysis was done using the Infinium Assay with the Illumina HumanOmniExpressExome Beadchip platform. B-allele frequency and log2R ratio were analyzed with Illumina Genome Studio V2009.2 software. DNA copy-number changes were prioritized using cnvPartition Plug-in v2.3.4. **Results:** SNP microarray analysis detected a clinically significant interstitial deletion of 4.2Mb of DNA from the long arm of chromosome 4 (4q31.22-4q31.3). This deletion includes *NR3C2*, which encodes for the MR. Parental chromosome studies to determine the mode of inheritance are currently pending. **Conclusions:** SNP microarray analysis identified a 4.2Mb deletion from chromosome 4q31.22-4q31.3 that includes *NR3C2* in an infant with hyponatremia and hyperkalemia, indicative of a diagnosis of PHA1. Genetic testing has been helpful in determining the diagnosis for this patient because initial laboratory values yielded a high PRA with low aldosterone. Molecular diagnostic confirmation will be done via Quantitative Multiplex Polymerase Chain Reaction of Short Fluorescent (QMPSF). Our patient is the first with a documented whole gene deletion of *NR3C2* as a cause for autosomal dominant PHA1. This report illustrates the clinical utility of SNP microarray as an initial genetic test in the evaluation of rare disorders, for which complex biochemical testing does not directly yield a clear diagnosis.

G06. Beyond the Standard Exome: A Roadmap to Comprehensive Clinical Sequencing of Disease Genes with High Homology

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Introduction: Exome sequencing is being implemented in clinical laboratories to enable comprehensive testing and accelerate diagnosis of genetic disease. However, limitations include inadequate coverage of many loci as well as difficulty interrogating genes with complex sequence contexts such as pseudogenes, genetic rearrangements, and high GC content. Complementary laboratory assays will need to be developed for the portion of the exome that is inaccessible through current NGS technologies. We report our work on one of these inaccessible genes, *STRC*, which is homozygously deleted in approximately 2% to 5% of individuals with autosomal recessive hearing loss, and whose analysis is complicated by a nearly identical pseudogene. Compound heterozygous cases with a deletion as well as small sequence variants have been reported in *STRC*. Therefore, comprehensive medical testing of this gene must include copy number assessment as well as non-standard sequencing assays. **Methods:** By parsing and compiling databases containing gene-disease associations, we have developed a comprehensive list of medically relevant genes (n = 4631). This list was cross referenced with a database of known pseudogenes, and one gene (*STRC*) was selected for developing a framework for analyzing these genes. Copy number assessment was performed on NGS data using VisCap, a novel tool tailored to targeted gene panels. Because NGS cannot correctly interpret the highly repetitive *STRC* coding region, we developed a long-range PCR assay that discriminates between gene and pseudogene and used this product as a template for standard Sanger sequencing. *STRC* copy number analysis and sequencing were performed on samples from patients without established genetic cause for their hearing loss despite prior NGS gene panel testing. **Results:** Of our list of medically relevant genes, 22% (1039/4631) have homology to other genomic loci. One such gene, *STRC*, is part of a tandem duplication on chromosome 15, which also contains a pseudogene, that is 99.6% identical across coding and intronic sequence. CNV analysis detected 11 *STRC* heterozygous deletions in our patient cohort. Of those, 6 (55%) were found to have a pathogenic substitution or small indel on the remaining allele. Biallelic *STRC* alterations were therefore established as the genetic cause of hearing loss in these patients. **Conclusions:** Exome wide genetic analysis is feasible but does currently not cover many medically relevant genes due to difficult sequence contexts. Our *STRC* results show that the development of ancillary assays to analyze these loci is critical for maximizing the clinical sensitivity of genetic testing.

G07. Replication of Panel of GWAS Candidate Genes/Loci for Myocardial Infarction in the Genetic Isolated Newfoundland Population

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Introduction: Genome-wide association studies (GWAS), have provided some promising achievements for a number of multifactor diseases including coronary artery disease (CAD). However, many of these GWAS candidate genes failed to been replicated in studies using different ethnic populations that indicates the variety of genetic modifiers among different ethnic populations. Additionally, the genetic heterogeneity in the studied population can reduce the sensitivity in detection of weak genetic effects and leads to false negative results in replication studies. The population of Newfoundland and Labrador (NL) is a well known genetic isolated population, and this population has a high prevalence of CAD in Canada.
Methods: As a part of our ongoing study, a panel of 15 genetic variants from 12 selected prior GWAS candidate genes/loci has been used to genotype 500 patients with myocardial infarction (MI) and 500 age- and sex-matched controls from the NL population by using the Sequenom's MassARRAY system. The further genotyping of selected SNPs from 9p21 locus in expanded population was conducted using Taq Man SNP genotyping technology on real-time PCR (ABI Prism 7000 Sequence Detection System). Odds ratios (OR) were calculated as a measure of the relative risk for MI and were given with 95% CIs. $P < 0.05$ (two-tailed) was considered statistically significant. Statistical power was calculated using QUANTO V1.2.3 software. **Results:** Among the 12 studied genes/loci, only the 9p21 locus (rs133049, rs10757274, rs238306, and rs238307) was successfully associated to the patients ($P < 0.000$ in all SNPs). This association was further confirmed in another study with enlarged sample size (1,000 MI patients and 1,000 controls) from the same population ($P<0.000$ in all SNPs). **Conclusions:** We, therefore, conclude that the 9p21 locus is a genetic susceptibility for CAD in the NL population. The failure of