GeNeXpreSS

Advancements in Genomic Diagnoses

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Variant interpretation in molecular autopsy (Scheiper-Welling et al., 2022)

The etiological diagnosis of sudden death is important for cascade testing of the relatives and preventive actions in individuals identified to be susceptible to sudden death. Molecular autopsy by next-generation sequencing (NGS) of the patients where detailed autopsy and histology has failed to identify the cause of death can be useful. With this objective this study investigated the genetic etiology of sudden cardiac death (SCD) among victims (age 1 year-50 years). Patients with acute drug abuse or myocardial infarction were excluded. NGS-based cardio panel focusing on genes associated with cardiac channelopathies and cardiomyopathies was used to study 56 deceased victims. Fifty-three rare protein-altering variants in 32 different genes were identified out of the 93 genes investigated. Among them, 17 variants were identified in genes with strong disease association while another 36 variants were identified in genes with low disease association. Most of the variants (51 out of 53 variants) found in this study were variants of uncertain significance (VUS) owing to lack of appropriate medical records and family history. VUS cannot be used for screening family members as this may lead to unnecessary anxiety and interventions. Seven cases had potentially actionable variants (two pathogenic and five potentially pathogenic). The large number of VUS detected by current data suggests the need for multidisciplinary team for analysis and guidelines for forensic NGS-based testing.

Detection of exon 7 deletion in *SMN1* through next-generation sequencing (Zhao et al., 2022)

NGS-based tests incorporating *SMN1* and *SMN2* copy number analyses are being employed widely for expanded carrier screening of couples, without the need for a second method validation. However, its performance has not been fully evaluated. In this study, 478 samples were re-analyzed with multiplex ligation-dependent probe amplification (MLPA), quantitative polymerase chain reaction (qPCR) and NGS for *SMN1* gene copy number. NGS could identify homozygous deletion as well as heterozygous deletion in the study. The sensitivity, specificity, and precision were all 100% which was higher than that of qPCR. Both NGS and qPCR methods showed 100% reproducibility for *SMN1* homozygous deletions, while for heterozygous deletions and non-deletion, NGS had higher repeatability relative to the qPCR method. With the same quality of DNA, the retest rate of NGS was the lowest (2.74%) as compared to MLPA and qPCR methods (6.69% and 5% respectively). In conclusion, NGS is a promising and fairly reliable method for expanded carrier screening for SMA caused by *SMN1* exon 7 deletion.

Reclassification of putative splicing variants through RNA diagnostics

(Bournazos et al., 2021)

In this study, the authors have devised standardized practices for PCR-based RNA diagnostics using clinically accessible specimens like blood, fibroblasts, urothelial cells, and biopsy tissue. A total of 74 families were recruited wherein a putative splicing variant was identified. Of the variants studied, 19% were those that affected the canonical GT-AG splice sites, 71% that affected the extended splice donor or acceptor sites, 27% were exonic variants and 2 were structural/copy number variants. PCR-based RNA assay was employed where total RNA was extracted from clinical accessible tissues followed by cDNA conversion. Probes were designed for splice junction or lack of splice junction e.g., intron retention followed by PCR and sequencing. This

assay helped in reclassification of 75% variants (58 cases) and also informed about mis-splicing events. Additionally, in two cases, the assay confirmed no evidence for mis-splicing which enabled reclassification into benign variants. For comparative evaluation of diagnostic utility, RNA-seq was performed in 19 cases studied by RNA-based assay which revealed that RNA-seq was non-diagnostic for 60% of cases because of low read depth.

Analysis of missense variants in the human genome reveals widespread gene–specific clustering

(Quinodoz et al., 2022)

The study investigated if clustering of missense variants could be a general feature of the entire human genome or is limited to a few specific loci, gene/ protein families or conserved domains. All pathogenic and likely pathogenic (PLP) and benign and likely benign (BLB) variants reported in ClinVar were extracted. These variants were used to build positional scores or MutScore by computing maximal allele frequency in gnomAD database of all PLP variants for every gene using a random forest approach. The study found that clustering of pathogenic missense variants occurs in almost half of all human genes, genome-wide, and about 18% of the clustering is highly delimited. Likewise, clustering also occurs in benign missense variants in approximately 20% of all genes associated with hereditary condition. They have also identified that PLP variants associated with dominant conditions are mostly identified in clusters while most recessive missense variations are dispersed along the protein sequence.

References

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