FUNCTIONAL ASSAY FOR ANALYSIS OF VARIANTS OF UNCERTAIN SIGNIFICANCE IN MAPLE SYRUP URINE DISEASE

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Introduction: Enhanced molecular characterisation using high-throughput sequencing has significantly improved patient diagnosis and management. However, the identification of variants of uncertain significance (VUS) limits its use for clinical decision making, such as prenatal diagnosis in subsequent pregnancies.

Case report: We report a female infant who presented with mild gross motor delay at 4 months and developed seizures with hypoglycaemia at 5 months. Plasma amino acid and urine organic acid findings were consistent with Maple syrup urine disease (MSUD), despite a normal newborn screening test for this condition. MSUD results in deficiency of branched-chain alpha-ketoacid dehydrogenase, responsible for leucine, isoleucine, and valine degradation, caused by biallelic pathogenic variants in *BCKDHA*, *BCKDHB*, or *DBT* genes.

Results: Molecular analysis in this patient identified two VUS, c.434-15_434-4del and c.365A>G (p. Tyr122Cys) in the *DBT* gene. Functional testing, in the form of leucine decarboxylation studies in fibroblasts revealed deficient function and mRNA splicing studies provided additional data that permitted variant reclassification and subsequent prenatal testing.

Conclusion: This case reinforces the need for specialist biochemical genetic laboratories to retain their ability to perform functional assays to aid the resolution of VUS.

ALK GENE REARRANGEMENT IN NSCLC BY NGS: AN EQA CASE REPORT

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Reliable detection of the anaplastic lymphoma kinase (*ALK*) gene rearrangement in non-small lung carcinoma (NSCLC) is crucial for selecting patients eligible for *ALK* tyrosine kinase inhibitor therapy such as crizotinib. Gene rearrangements are commonly identified using fluorescence in situ hybridisation (FISH) and immunohistochemistry (IHC). In the past decade, next generation sequencing (NGS) is gradually being introduced into clinical laboratories for its ability to screen for multiple molecular markers of NSCLC in a time efficient manner.

An external quality assurance program was introduced in 2020 to monitor laboratory performance in diagnostic interpretation of *ALK* gene rearrangement analysis by FISH, IHC and molecular

techniques. Forty-three laboratories were enrolled in this program and seven laboratories (16%) performed molecular detection of *ALK* fusion variants by NGS or reverse transcription PCR (RT-PCR). There was a 60% discordance within the molecular results reported in this survey where laboratories failed to detect the presence of an *ALK* fusion variant. In summary, we describe the outcome of an investigation to determine the exact *ALK* fusion variant in this case.

DOG1 EXPRESSION IS IN COMMON HUMAN TUMOURS: A TISSUE MICROARRAY STUDY ON MORE THAN 15,000 TISSUE SAMPLES

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DOG1 (Discovered on GIST1) is a voltage-gated calcium-activated chloride and bicarbonate channel that is highly expressed in interstitial cells of Cajal and in gastrointestinal stromal tumours (GIST) derived from Cajal cells. To systematically determine in what tumour entities and normal tissue types DOG1 may be further expressed, a tissue microarray (TMA) containing 15,965 samples from 121 different tumour types and subtypes as well as 608 samples of 76 different normal tissue types was analyzed by immunohistochemistry. DOG1 immunostaining was found in 67 tumour types including GIST (95.7%), oesophageal squamous cell carcinoma (31.9%), pancreatic ductal adenocarcinoma (33.6%), adenocarcinoma of the papilla Vateri (20%), squamous cell carcinoma of the vulva (15.8%) and the oral cavity (15.3%), mucinous ovarian cancer (15.3%), oesophageal adenocarcinoma (12.5%), endometrioid endometrial cancer (12.1%), neuroendocrine carcinoma of the colon (11.1%) and diffuse gastric adenocarcinoma (11%). Low level-DOG1 immunostaining was seen in 17 additional tumour entities. In conclusion, our data identify several tumour entities that can show DOG1 expression levels at similar levels as in GIST. Although DOG1 is tightly linked to a diagnosis of GIST in spindle cell tumours, the differential diagnosis is much broader in DOG1 positive epithelioid neoplasms.

VILLIN EXPRESSION IN HUMAN TUMOURS: A TISSUE MICROARRAY STUDY ON 12,429 TUMOURS

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Villin protein is part of the brush border of the apical membrane of epithelial cells. Villin is used as an immunohistochemical marker for colorectal and gastrointestinal neoplasms although other tumour entities can also express villin. To comprehensively determine villin expression in normal and neoplastic tissues, a tissue microarray containing >14,000