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# Abstracts of the 2nd AMP Europe Congress:
## Clinical Genomics: Beyond the Somatic Mutation

### The Association for Molecular Pathology

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**Milan, Italy**

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**Selected Oral Abstracts**

**Selected Genetics/Inherited Conditions Abstracts**

**01-OR01. Benchmarking an Artificial Intelligence Method for Fast Diagnosis of Rare Genetic Disease**

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**Introduction:** Evidence is mounting that genome sequencing should be performed as a first-tier test for children in multiple pediatric settings, especially for those in the intensive care unit. As more children are sequenced, the pressures to increase the diagnostic rate and the time to result, while reducing the cost of the process, are amplified. Interpretation of whole-genome or exome variants to diagnose rare genetic diseases continues to be a major bottleneck, as it often consists of time-consuming iterative variant filtering coupled with evidence review for large lists of candidate variants. We showed previously that VAAST and PHEVOR, tools used in tandem to prioritize variants given patient phenotype descriptions in HPO terms, reduced review to 20 variants for 75% of 450 cases with positive findings from the Genomics England 100,000 Genomes Project. **Methods:** Here we benchmark GEM, a novel artificial intelligence-based method, which integrates the outputs of VAAST and PHEVOR with knowledge from OMIM, gnomAD, and ClinVar databases to quickly identify disease-causing variants from genomes. GEM is robust to common sequencing artifacts and cryptic ancestry, predicts consanguinity and inheritance mode, and can take advantage of automated deep phenotyping. GEM outputs a short list of likely disease genes and only returns candidates when a Bayes factor score supports evidence of genetic causality, therefore preventing lengthy and sometimes unnecessary case reviews. **Results:** We are performing a benchmarking study of GEM by analyzing 200 solved rapid whole genome sequencing cases of seriously ill children from the Rady Children's Hospital NICU. In our preliminary analysis of 56 cases, we show that GEM ranks previously identified disease genes as the top candidates for 82% of cases, and within the top 5 or 10 candidates for 97% and 100% of cases, respectively. The mean number of candidates returned per case is 3.7, with a median and mode of 1. Importantly, automated deep phenotyping by NLP of clinical notes did not degrade the ranking, and in fact improved it despite returning large numbers of HPO terms (e.g., in one case up to 650). Additionally, we analyzed 14 previously unsolved cases where GEM returned new potential findings for 2 cases, without yielding false leads for the others. **Conclusions:** GEM significantly simplifies and improves disease-causing variant prioritization over prior methods, substantially reducing genome interpretation time in the diagnosis of monogenic disease, and could allow cost-effective, automated reanalysis of undiagnosed cases over time.

**01-OR02. Characterization of a Rare/Varying Clinical Consequence of *CFTR* Variants *W57G*/*A234D CFTR* Genotype and Theratyping Using Rectal Organoids**

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**Introduction:** Over 2,000 different variants in the *CFTR* gene have been described: not all of them cause cystic fibrosis (CF). This study focused on a CF patient carrying the very rare CF-causing *CFTR* variant *W57G* (c.169T>G), described in 10 patients in the *CFTR2* database and recently found unresponsive to ivacaftor in a human bronchial epithelial cell model, in trans with the *A234D* (c.701C>A) missense variant that is not described in the above-mentioned database. The complex allele *A234D-I1027T* has been previously reported by Seia *et al.* (www.cysticfibrosisjournal.com/article/S1569-

**09-P46. Comparison of Single Gene Testing and NGS Multiplex Panels in Non-Small Cell Lung Carcinomas: An Institutional Experience**

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**Introduction:** Recent advances in the armamentarium against non-small cell lung carcinoma (NSCLC) benefit patients with advanced disease and prompted reflex molecular testing in most institutions. Initially, single gene testing (SGT) was performed (i.e., *EGFR* testing; if wild-type result reflex to *ALK1, ROS1, RET, KRAS*). However, the limited amount of tissue in cytology or small biopsies sometimes precluded molecular testing for all the genes with available targetable alterations, potentially delaying optimal treatment. With the advent of novel genetic alterations being discovered and next-generation sequencing (NGS) becoming broadly available, the general trend was switching from SGT to NGS multiplex panels. This was emphasized by the subsequent release of CAP/IASLC/AMP "Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors" in 2018. Few studies have compared the impact of switching from SGT to NGS panels in NSCLC. **Methods:** We retrospectively looked at NSCLC cases in our institution for which SGT or NGS panels were used. A total of 162 cases were retrieved (from January 2018 to July 2019) that underwent molecular testing by either SGT or NGS. Cases with insufficient tissue for molecular testing (QNS) were recorded. Comparison between the 2 groups was performed using Bayesian logistic regression with a diffuse prior. **Results:** The mean age of the patients at the time of diagnosis was 69.0 years (female:male ratio 1.4:1). A total of 16 cases showed *EGFR* alterations and 28 were positive for *KRAS* mutations. SGT was performed on 100 (61.7%) cases, of which 11 (11%) were QNS. Of these, 63.6% (7/11) were QNS for EGFR testing, whereas 4 were QNS for ALK testing. Sixty-two (38.3%) cases were tested by NGS; only 1 (1.6%) was QNS. The odds of cancelling a test as a result of QNS for SGT is statistically higher compared to testing by NGS panels (odds ratio: 4.67 [95% CI: 2.66-7.41]). **Conclusions:** SGT is more likely to be cancelled due to QNS than testing using multiplex NGS panels. To maximize molecular testing on small tissue biopsies and/or cytology specimens, multiplex genetic sequencing panels are preferred over multiple SGT, in accordance with the molecular testing CAP/IASLC/AMP Guideline.

**09-P47. Exosomes of Glioblastoma Present Higher Molecular Variation than a Tumor Primary Cell Line**

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**Introduction:** Increasing evidence indicates that extracellular vesicles (EVs) secreted from tumor cells play a key role in the overall progression of the disease state. EVs such as exosomes are secreted by a wide variety of cells and transport a varied population of proteins, lipids, DNA, and RNA species within the body. Gliomas including glioblastoma (GBM) are the most common primary malignant brain tumors. Glioma EVs including exosomes have biological effects (e.g., immunosuppression) and contain tumor-specific cargo that could facilitate liquid biopsies. Recent articles show the potential of cfDNA in the therapeutic analysis of glioblastoma. In this report we highlight the potential of DNA EV next-generation sequencing (NGS) assay in glioma. **Methods:** Eleven patients from A.U.O. "Sant´Andrea", Neurosurgery Division, Sapienza University, Rome, NESMOS Department, were enrolled in this study. Here we describe a new custom QIAGEN NGS assay technique to analyze DNA plasma exosomes in glioma patients in correlation to DNA extracted from cryostat section. Here we report the analysis of the *H3F3A, IDH1, IDH2, TERT, CDKN2A, TP53, NF1*, and *ATRX* genes with a QIAGEN GeneReader custom panel with 0.5% variant allele frequency (VAF). **Results:** All the genes were analyzable in 40% of EVs sampled. All had reads with average quality of >25 and a percentage of base positions in regions of interest with UMI coverage ≥200x in a minimum of 75%. Our data show that *NF1* had more pathogenic mutation in exosome sample than in matched cryostat section (3 ± 0.7 versus 2 ± 1.04). Interestingly, the exosomes showed a high number of variants detected than in matched cryostat section (51.67 ± 19 versus 21.42 ± 14, p value <0.05). **Conclusions:** Our report on DNA glioma EVs highlights the prognostic-diagnostic potential of the NGS assay in the glioblastoma patients.