remain unclear. To study whether the V-type H^+-ATPase (VA) plays a role in CAPA inhibition, we performed fluid secretion assays in MTs treated with diuretics and bafilomycin, a known VA inhibitor. Bafilomycin significantly inhibited DH_{31}-stimulated fluid secretion 30 min post treatment compared to diuretic controls ($p<0.05$, n=22). Similar, however, delayed responses were seen in 5HT-stimulated MTs, while no affect was observed in DH_{44}-stimulated secretion. An indirect way to measure whether CAPA inhibits VA activity was to measure the pH of the secreted fluid from diuretic-stimulated MTs treated with CAPA. In DH_{31}-stimulated MTs supplemented with CAPA, there was an immediate significantly higher pH at 40 min, increasing up to 7.73±0.038 compared to control, 7.56±0.038 ($p=0.0007$, n=20). The pH of 5HT-stimulated MTs treated with CAPA was seen to significantly increase up to 7.75±0.061 ($p=0.03$, n=10) at the 60 min mark, in agreement with the delayed response previously seen. Unlike the effects observed with DH_{31} and 5HT, CAPA did not alter the pH of the secreted fluid in DH_{44}-stimulated MTs. Alkalization of the secreted fluid in response to CAPA suggests inhibition of the proton pump, which may lead to constrained cation entry across the apical membrane of the MTs. To understand Na^+/K^+-ATPase (NKA) and VA activity in response to CAPA, we performed activity assays in diuretic-stimulated MTs. Adult female MTs treated with DH_{31} resulted in an increase of both NKA and VA activity compared saline controls. As expected, MTs incubated with both DH_{31} and *Aedae*CAPA-1 had a lower NKA and VA activity ($p<0.05$) resulting in activity levels comparable to saline control levels. The results thus far could suggest a novel mechanism for CAPA inhibition, blocking the VA to hinder fluid secretion. Investigating the pathway of CAPA inhibition and its role in countering diuresis will help provide a deeper understanding of the critical process of diuresis and its signaling mechanism.

Genetics and Development (including Gene Regulation)

FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

Comprehensive and Cost-Effective Strategy for Genetic Screening of Congenital Adrenal Hyperplasia (CAH) in India

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Background: With substantial challenges in molecular analysis of 21 hydroxylase deficiency and lack of studies in extended panel of genes implicated in CAH, genetic diagnosis is largely unavailable and unaffordable in India. Therefore, we aim to develop a cost-effective screening strategy in CAH using Allele Specific PCR and Targeted Next-Generation Sequencing (NGS). **Methods:** Long range PCR and restriction digestion were utilized to specifically amplify the CYP21A2 gene whereas multiplex PCR was used to amplify CYP11B1, CYP17A1, CYP19A1 and POR genes. In house developed Allele Specific PCR (ASPCR) for 8 hotspot mutations in CYP21A2 gene and targeted NGS for five genes was carried out. The results were validated using Sanger sequencing and MLPA. **Results:** Of the 50 patients suspected for 21 hydroxylase deficiency, 64% (n=32) were of Salt Wasting phenotype (SW), 30% (n=15) with Simple Virilizing (SV) phenotype and 6% (n=3) of the study population were suspected for non-classical (NC) CAH. The mutation positive rate of ASPCR was 86% (n=43). Seven patients carried more than two biallelic mutations indicating smaller gene conversions. The predominant mutation identified among the study subjects was I2G splice variant in SW phenotype (38%) and I172N in the SV phenotype (41%). Based on the Long range PCR amplification and restriction digestion we identified one patient with large gene conversion and one patient with large 30kb deletion. These results were confirmed with MLPA.

Additionally, utilizing the Targeted NGS we identified five patients with CYP21A2 variants (two patients with novel variants c.1274G>T, c.17_18delTG and two other variants in three patients - c.1451G>C, c.143A>G). We also identified a CYP19A1:c.1142A>T gene variant in a patient who was initially suspected for 21 hydroxylase deficiency. Out of six patients with 11 beta hydroxylase deficiency four patients were positive for homozygous CYP11B1 variants (c.1201-1G>A, c.1200 + 1delG, c.412C>T) and two patients with compound heterozygous variants (c.1024C>T and c.1012dupC, c.623G>A and c.412C>T).

Discussion: Utilizing the novel allele specific PCR followed by NGS we identified a total of 96% (48/50) of 21 hydroxylase deficiency patients with homozygous or compound heterozygous mutations and 2% (2/50) were positive for single heterozygous variant in CYP21A2 gene. ASPCR followed by multigene targeted NGS assay for genetic screening in CAH has shown to be a sensitive and specific strategy established in a clinical setting. To best of our knowledge this is the most cost-effective and comprehensive multigene screening carried out in India.

Genetics and Development (including Gene Regulation)

FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

Differential Activity and Utilization of Glucocorticoid Receptor Binding Sites Yields Transcriptional Heterogeneity

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