Androgen Insensitivity Syndrome (AIS) is an X-linked genetic disease and it is the most common cause of 46,XY DSD. It is divided into 3 phenotypes: complete (CAIS), partial (PAIS), and mild (MAIS). To analyse the landscape of AR variants in AIS we collected all AR variants reported among AIS in the literature (Pubmed, EMBASE, Medline) and websites (ensemble, HGMD, ClinVar). They were analyzed according to phenotype, exon location, domain, amino acid (aa) conservation, sex assignment, external genitalia virilization (EMS score), molecular and functional studies. Conservation analysis of the AR were performed using CONSURF plataform. To test our hypothesis that non-synonymous AR variants could also impact on splicing, we used both ESEfinder and Human Splicing Finder 3.1. We founded 901 individuals with AIS: CAIS = 565 (62.7%); PAIS = 282 (31.3%); and MAIS = 54 (6%). They had 465 different AR variants: CAIS = 290 (62.3%); PAIS = 135 (29.1%); and MAIS = 40 (8.6%). Among MAIS and PAIS, most variants were at LDB domain (22 out 40 = 55% and 84 out 135 = 62.2%, respectively) whereas they were at NTD domain among CAIS (129 out 290 = 44.5%). Most were missense (81%). However, small indels (11%), nonsense (3%), splicing sites (4%) and large deletions (1%) were all reported. Non-synonymous AR variants accounting for 60%, 96%, and 100% of CAIS, PAIS, and MAIS, respectively. Synonymous AR variants were rarely found (n=3). In 81% only the AR sequencing was performed. The remaining was detected by WES (18%) or WGS (1%). Deep intronic variant was detected in PAIS (n=1) while variants in the 5'UTR of the AR gene in both PAIS and CAIS (n=2). Most AR variants were located at conserved aa (78%), but AR variants at non-conserved aa were more frequently indels (p<.01). Functional studies were found in 38%, mostly showing reduced AR expression. Among PAIS, 48% (n=134) were assigned as male at birth. The median EMS was 5 (95% CI, 5-7) in those assigned as male while it was 3.2 (95% CI, 2-6) in those assigned as female (p<.01). The median of EMS score was lower in variants at NTD domain (2.8, 95% CI, 0-7). We identified 34 AR variants causing more than one AIS phenotype (mostly CAIS and PAIS) and 6 AR variants causing all of three AIS phenotypes. *In silico* analysis suggests potential to disrupt normal AR splicing in 18 (53%) by creating new acceptor or donor splicing sites (n=11) or exonic splicing signals (n=7). More severe AR variants are related to CAIS. Most AR variants were reported only based on AR sequencing. Therefore, the functional pathogenicity of these variants remains unclear. Further studies including WGS could help to expand the molecular diagnosis of AIS. There is phenotype variability in AIS. So, sex assignment of patients with PAIS cannot be based on a specific identified AR gene mutation. There is potential to alter splicing among non-synonymous AR variants, which could be an explanation for phenotype variability in AIS.

Genetics and Development (including Gene Regulation)

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

Universal Multi Gene Panel Testing For Individuals With Pheochromocytomas And Paragangliomas

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Background: Pheochromocytomas (PCCs) and paragangliomas (PGLs) (PPGLs) are a genetically heterogeneous entity, with roughly 25-40% of cases found to harbor a pathogenic or likely pathogenic germline alteration. Existing practice guidelines advocating for the use of a sequential gene testing strategy to identify individuals with hereditary PPGL are driven by the presence of specific clinical features and predate the routine use of multigene panel testing (MGPT). Here we describe results of MGPT for hereditary PPGL in a clinically and ancestrally diverse cohort from a diagnostic laboratory. **Methods:** Demographic and clinical information of individuals undergoing targeted MGPT for hereditary PPGL were collected from test requisition forms and supporting clinical documents provided by the ordering clinician and retrospectively reviewed. Individuals underwent MGPT of 10-12 genes depending on test order date. From August 2013 through May 2015, 560 individuals had targeted MGPT that included 10 genes (*NF1, MAX, SDHA/B/C/D/AF2, RET, TMEM127,* and *VHL)*, and from May 2015 through December 2019, 1167 individuals had panel testing of 12 genes due to the addition of *MEN1* and *FH*. **Results:** Overall, 27.5% of individuals had a pathogenic or likely pathogenic variant (PV), 9.0% had a variant of uncertain significance, and 63.1% had a negative result. Out of all PVs, most were identified in *SDHB* (40.4%), followed by *SDHD* (21.1%), *SDHA* (10.1%), *VHL* (7.8%), *SDHC* (6.7%), *RET* (3.8%), and *MAX* (3.6%). PVs in *FH*, *MEN1*, *NF1*, *SDHAF2*, and *TMEM127* collectively accounted for 6.5% of PVs. Clinical predictors of a PV included extra-adrenal location, diagnosis before the age of 45 years, multiple tumors, and positive family history (fhx) of PPGL. Affected individuals with a fhx of PPGL were the most likely to have a PV (70.6% of individuals with PCC + fhx; 85.9% of individuals with PGL + fhx). The positive rate in nearly all clinical subgroups even without predictors of a PV remained over 10%, including individuals with a single tumor (PCC = 16.7%; PGL = 46.7%) and those without a fhx (PCC and negative fhx = 15.8%; PGL and negative fhx = 43.7%). Restricting genetic testing of hereditary PPGL to only *SDHB/C/D* genes misses a third (31.8%) of individuals with PVs. Among individuals with PVs in syndromic genes, over half (41.5%) did not have any additional syndromic features beyond PPGL reported by the ordering clinician. **Conclusion:** Our data demonstrate a high diagnostic yield in individuals with and without established risk factors, a low inconclusive result rate, numerous individuals with syndromic PVs presenting with isolated PPGL, and a substantial contribution to diagnostic yield from rare genes when included in testing. These findings support updating practice guidelines to incorporate universal testing of all individuals with PPGL and the use of concurrent MGPT as the ideal platform.