Whole Exome Sequencing (WES) reveals oligogenic gene mutations in a case of Combined Pituitary Hormone Deficiency (CPHD)

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### INTRODUCTION AND OBJECTIVE

CPHD is characterized by GH and at least one other pituitary hormone deficiency. Pathogenic variants in genes expressed in the developing head, hypothalamus, and/or pituitary cause CPHD. To date around 30 genes have been identified to be related to CPHD, however the aetiology of 85% of the cases remains unknown. Whole Exome Sequencing (WES) enables parallel searching for pathogenic variants of CPHD in targeted known gene panels as well as the identification of novel genes related to CPHD thus allowing genetic diagnosis, prognosis and genetic counseling.

The scope of this study was to identify the molecular defects of a patient with CPHD employing Whole Exome Sequencing (WES).

### PATIENT

A newborn boy (46, XY) delivered by Caesarean section at 37<sup>1/2</sup> weeks due to intrauterine growth restriction with a birth weight of 2020 g, presented with refractory hypoglycemia and mild hyponatremia. On physical examination, he had micropenis with bilaterally pulpable small testes. Endocrinological work up revealed secondary hypothyroidism, secondary adrenal insufficiency and hypogonadotropic hypogonadism (HH). MRI scan of the hypotalamic pituitary region depicted hypoplastic anterior pituitary and ectopic posterior pituitary lobe with absence of pituitary stalk.

### METHODS

WES was carried out on an Ion Torrent S5 platform. The data was aligned to the hg19 reference with Torrent Mapping Alignment Program and was annotated by the Ion Reporter software and the ANNOVAR using VarFT software. Employing WES, 18921 genes were sequenced with a coverage of 90.72% and >20x depth of reads. A total of 38348 variants were detected in 12459 genes. An in silico panel of 79 genes related to CPHD was employed to search for variants with MAF values <1% and depth of reads >20x. Phenotype based gene analysis was carried out employing Human Phenotype Ontology (HPO). The pathogenic variants selected were verified by Sanger sequencing in our patient and his parent.

### RESULTS

After WES data analysis (Fig.1), three heterozygous variants were detected to be related to the patient’s phenotype in three genes: the paternally inherited pathogenic stop codon variant p.R73X of the GNRH1 gene, and two maternally inherited variants of uncertain significance (according to ACMG criteria) the p.A42P of the BMP4 gene and the p.Q32E of the SRA1 gene.

### CONCLUSIONS

WES facilitated the identification of 3 variants related to patient’s phenotype in BMP4, GNRH1 and SRA1 genes. BMP4 plays significant role in early organogenesis, pituitary development and function and thus represent plausible candidate for mutational screening of CPHD patients. The SRA1.p.A42P variant has been previously described in a patient with tooth agenesis, however another heterozygous BMP4 variant, p.R300P, has been reported in a case with CPHD and hypoplastic pituitary gland. The pathogenic variant GNRH1.p.R73X has been previously described in a patient with HH. SRA1 (Steroid Receptor Activator) is a functional ncRNA which among its other functions regulates steroid receptors-dependent gene expression (Fig.3). The variant SRA1.p.Q32E has been previously identified in a patient with HH and could probably explain the secondary adrenal insufficiency of our patient, since SRA1 regulates SF1 target gene expression by functioning as a coactivator in association with DAX1. GNRH1 interacts with Thyrotropin releasing hormone (TRH) (Fig.2), whereas SRA1 coactivates among other receptors, the thyroid hormone receptor and thus, we might hypothesize that the variants of these two genes might be the cause of the secondary hypothyroidism of our patient.

We speculate that a synergistic action of these gene pathogenic variants may underlie our patient’s phenotype.

### REFERENCES