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^{+3} weeks due to intrauterine growth restriction with a birth weight of 2200 g, presented with refractory hypoglycemia and mild hypotonia. 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 hypothalamic 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 VarAFT 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.

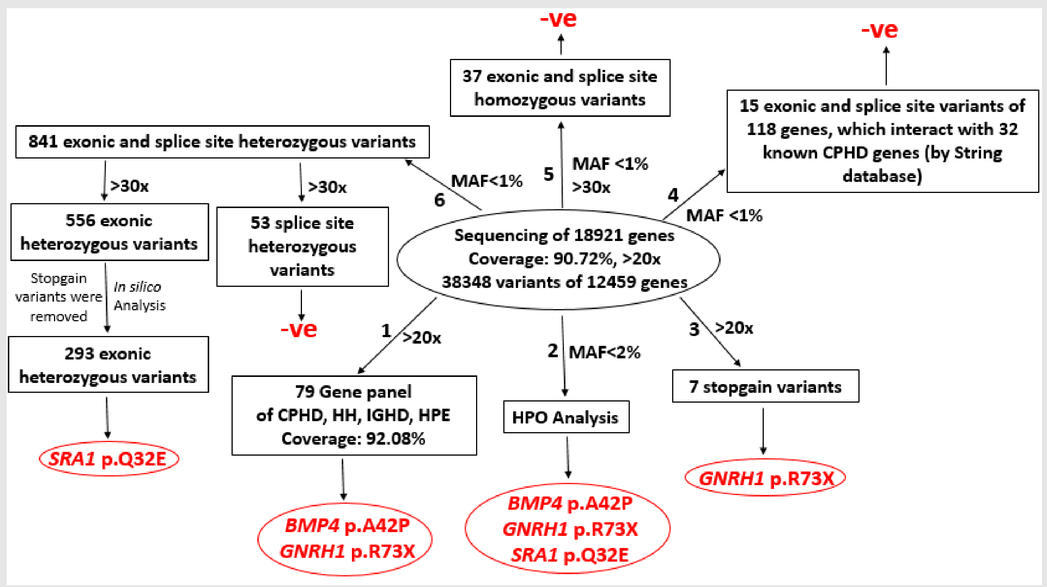

Figure 1. Analysis pipeline

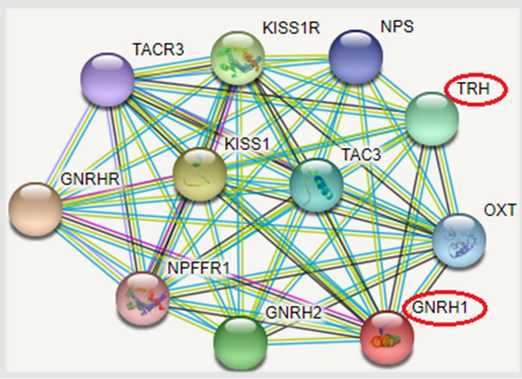

Figure 2. String network interaction of GNRH1

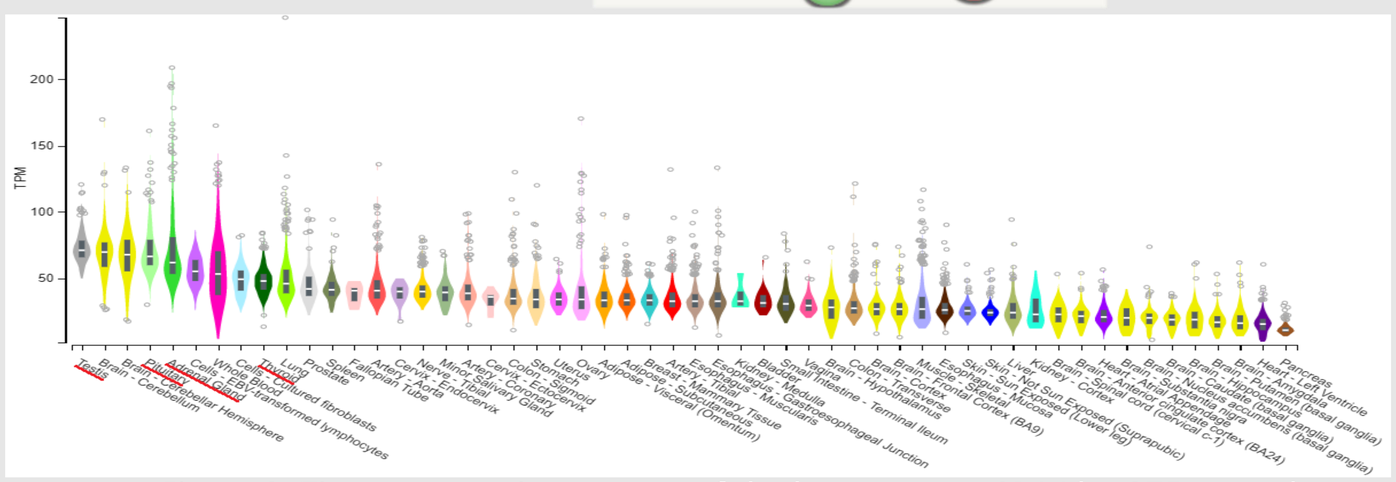

Figure 3. Tissue expression pattern of the human gene *SRA1* by GTEx portal

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 ***BMP4*;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 regulate steroid receptors-dependent gene expression (Fig.3). The variant ***SRA1*;p.Q32E** has previously been 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.

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58ESPE P1-118
Pituitary, neuroendocrinology and puberty
AMALIA SERTEDAKI
Poster presented at: ESPE 2018 VIENNA
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